IMPACTS OF DEER AND EARTHWORMS ON UNDERSTORY FOREST PLANTS

A Dissertation
Presented to the Faculty of the Graduate School
of Cornell University
in Partial Fulfillment of the Requirements for the Degree of
Doctor of Philosophy

by
Annise Marie Dobson
May 2018

IMPACTS OF DEER AND EARTHWORMS ON UNDERSTORY FOREST PLANTS

Annise Marie Dobson, PhD
Cornell University 2018

ABSTRACT

Northeastern North American forests experience a myriad of stressors, influencing their capacity to sustain diverse communities, provide ecosystem services and replenish valuable timber resources. Expansions of non-native earthworms and native white-tailed deer populations have occurred simultaneous with other stressors, but conservation and management mandates are focused on individual threats. Here, we provide a mechanistic explanation of the individual and combined effects of deer and earthworms on forest understory plant species using experimental plantings in a 2 x 2 factorial design. We seek to understand what makes many species decline under these altered forest conditions, and to test the viability of restoring plant communities. First, we assessed earthworm impacts on cycling of a broad spectrum of nutrients. We found earthworms are associated with lower soil P, but higher concentrations of other nutrients in the A horizon, including Ca, Mg, K and S. Despite this, we saw little rooting in the A horizon of earthworm invaded plots. This could be due to the stressful rooting conditions in the surface soil of the A horizon created by earthworm activity. If non-native plants that have coevolved with earthworms are able to access these nutrient-rich pools that are largely untapped by the background native vegetation, they may proliferate in earthworm-invaded forests. Of the native species were assessed, successful species were able to incorporate additional Ca into their tissues, and maintained consistent tissue P despite earthworm-associated depletions in soil. Species that declined could not capitalize on higher soil Ca in earthworm invaded plots, and had lower concentrations of P in roots and leaves. For the next two studies, we used transplant experiments of species with a breadth of growth forms to standardize species pools. We

explored indirect methods of deer and earthworm impact on fine roots, mycorrhizal associations and soil nutrients. We found earthworms and/or deer decreased % colonization by arbuscular mycorrhizal fungi (AMF) in one species (*Quercus*) but not others, negatively affected soil nutrient concentrations and pools and increased or decreased the proportion of fine roots relative to total root length. However, this did not reliably translate to changes in seedling survival or biomass. Finally, we followed transplants of 20 native understory species over four to six years. Initially, seedlings of most species had poorer establishment in earthworm invaded plots, but by the end of the experiment, earthworms benefited 13 of 20 species and negatively affected five. Earthworm impacts on seedling survival was largely decoupled from impacts on growth and reproduction, with most species performing better in earthworm-invaded plots. Deer limited most species' growth, reproduction, and (to a lesser degree) survival, including tall, non-palatable species. Survival of species with high foliar nitrogen concentrations were slightly diminished in the presence of both deer and earthworms. Despite lower survival of some species in earthworm-invaded plots, we were successful in establishing shade-intolerant species into forests. This suggests that after initial changes to the forest floor from earthworm invasion have stabilized, restoration of the vegetation can be successful if it is paired with deer management.

BIOGRAPHICAL SKETCH

Annise Dobson was born in Pembroke, Ontario, Canada on November 4th, 1987. Free to explore the farm's "100 acre wood," she has always felt most at home in the forest. She attended Cobden District Public School and Opeongo High School in Douglas, Ontario. Her first research experience was at the Deep River Science Academy, examining the effects of buried nuclear material on photosynthesis at the site of a nuclear facility in Chalk River. She attended McGill University, receiving a Bachelor of Agricultural and Environmental Science, double-majoring in Environmental Biology and (Molecular) Botanical Science. Her research focused on chemical signaling crosstalk between plants and insect herbivores. She worked with Tanya Copley and Dr. Jacqueline Bede, who taught her how to do research, but more importantly, how to be a researcher. She took several field courses in Quebec and one in Barbados, where she learned how to be dynamic and creative with fieldwork when it inevitably does not go as planned. In the summers, Annise worked at OWL Rafting on the Ottawa River and tree planting in remote parts of Canada. She completed her Master's of Science in the Department of Natural Resources at Cornell University under the advisement of Dr. Bernd Blossey. Her research focused on the effects of multiple stressors on understory forest plant communities, and she has continued this work as a PhD student. She found great pleasure in learning to read the human and natural history of the forests of New York state from the forest floor, stone fences, species assemblages and architecture of the trees. In graduate school, she had the opportunity to teach a diverse set of courses including the laboratory-based Investigative Biology, DNA, Genes and Genetic Diversity, Introductory Ecology and Field Biology. It was during these teaching opportunities that Annise discovered her passion for finding clear ways to communicate complex ideas. In her final semester of teaching, Annise received an Outstanding TA award from the Undergraduate Biology Department.

ACKNOWLEDGEMENTS

My advisor Dr. Bernd Blossey has been my biggest cheerleader, and his encouragement has given me the confidence to pursue the questions that drive me, no matter how big the challenge. Dr. Tim Fahey has guided me with research design, local and natural history, teaching and making sense of quagmires of results. Dr. Taryn Bauerle expanded my interests in root biology, provided me with technical help and lab space, and has been an inspiring mentor. Dr. Anurag Agrawal challenged me to think deeply about the mechanics of an experiment, and encouraged me to see the beauty in statistics. For all of their support, advice and dedication I am extremely grateful.

The nutrient research was done in close collaboration with Dr. Justin Richardson, whose enthusiasm and teaching I will always appreciate. The fern experiments were spearheaded by Audrey Bowe, who has provided incredible support throughout these experiments. I would like to thank Brian Flannigan and Neil Mattson for support analyzing roots, and Alexis Heinz, Marie Zwetsloot, Annika Kreye, Mark Brundrett, Jon Gonzales and Juana Munoz Ucros for help with mycorrhizal staining, Greg McElwee and the Derry Lab for laboratory assistance with ICP-OES and ICP-MS analyses and Kimberly Sparks with the Cornell Stable Isotope Lab (COIL).

I would like to thank everyone who has helped me in the field, including Wade Simmons, Juan Pablo Jordan, Elizabeth Simpson, Shauna-kay Rainford, Jeremy Dietrich, Darragh Hare, Marcus Franklin, Ethan Keller, Jaime Ortiz, James Burtis and Robert Koch. Andrea Dávalos, Francoise Vermeylen, Stephen Parry and Erika Mudrack have helped me enormously with my experimental design and statistics, and Krissy Boys, Harlan Brumsted and Robert Wesley shared their immense local natural history knowledge. I would like to thank Peter Ducey for assistance with earthworm identification and the Cornell Soil and Water laboratory

and the Fahey/Yavitt laboratory for their guidance and use of their laboratory facilities. Victoria Nuzzo shared her vast botanical knowledge, provided seeds and seedlings, and guided and inspired me in almost every step of my research. This project was funded through a Cornell University hatch grant (to BB), the Cornell Graduate School and the Andrew Mellon Foundation. Friends from the White House, my Femina Sci and W&W sisters, and the DNR community have supported me and encouraged me to grow throughout my years in graduate school. I'd like to thank my sisters, Emma and Loreena, and my parents, Grant and Dorothy, who taught me to be curious and work hard. I'm incredibly thankful to Ben Machta for his love, support, and unwavering belief in me. Finally, I would like to thank the people of the Haudenosaunee Confederacy, on whose traditional territory this research was conducted.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

Chapter 1

INTRODUCTION

The forest understory is low in aboveground biomass relative to canopy trees, but plant species in the understory are critical to above and belowground food webs, nutrient cycling, and carbon sequestration (Gilliam 2014; Bohlen et al. 2004b)). Understory plant communities hold up to 90% of plant biodiversity in temperate forests and filter which species reach the canopy by determining which seeds and propagules can establish (Dorning and Cipollini 2006; Royo and Carson 2008; Gilliam 2014). These plant species have evolved unique strategies to cope with herbivore browse and limitations in light, temperature and moisture. Many of these strategies rely on the physical, chemical and biotic conditions found on the forest floor. From at least the Wisconsinan glaciation, organic matter (OM) inputs in northeastern North American forests have exceeded uptake and loss, leading to a buildup of OM on the forest floor. As a result, deep organic horizons developed, creating an aerated microhabitat and acting as a buffer to moisture and temperature fluctuations (Larson et al. 2010; Eisenhauer et al. 2012). The stability and aeration in the organic horizon creates ideal growing conditions for fine roots and a network of fungal hyphae. This supports a diverse food web, which is the interface of below and above-ground interactions. These forest soils have historically been stratified, and nutrient cycling has been tightly coupled with forest plants, mycorrhizal symbionts, and microorganisms to maximize nutrient retention in shallow horizons through close coupling of decomposition and uptake processes (Leonard and Field 2004; Suarez et al. 2004; Frelich et al. 2006; Gilliam 2007; Hale et al. 2008).

Recently, these forests have been experiencing multiple stressors such as species invasions, land-use change, nitrogen deposition, high deer browse pressure and climate change, threatening this closely coupled system (Côté et al. 1995; Dávalos et al. 2014). The emergence of

these stressors co-occurred with disruption of the forest floor and the understory communities that rely upon it (McKinney and Lockwood 1999; Wiegmann and Waller 2006). However, because these stressors are often co-occurring, identifying which are the drivers and which the passengers of change presents a unique challenge (MacDougall and Turkington 2005; Didham et al. 2005). In this dissertation, I parse out several mechanistic hypotheses to understand individual and combined effects of deer and earthworms on understory plant communities.

Chapter 2 explores earthworm impacts on plant-soil nutrient dynamics. We use this framework to understand how perturbations to nutrient dynamics determine plant species performance. Community comparisons through time and herbarium records have identified "winners" and "losers", with sensitivity to disturbance as a key attribute in their decline (Wiegmann and Waller 2006; Roberts and Gilliam 2014). While this designation is helpful in identifying species of most conservation need, it has limited value in proposing conservation and management strategies. Chapter 3 tests three possible indirect hypothesis about earthworm and deer influence on understory plants: changes to nutrient cycling, disruption of plant-arbuscular mycorrhizal fungi (AMF) mutualisms and suppression of fine roots.

Chapter 4 follows the performance of seedlings originally set up in my Masters research, including herbs, ferns, graminoids and woody plants. In my Masters, with one growing season of young seedlings, I observed only minimal deer effects (Dobson and Blossey 2015). In the first growing season, earthworm effects were broadly negative, even for growth forms thought to benefit from earthworm activity (Hale et al. 2006b; Holdsworth et al. 2007a). Here, we take a longer-term view of deer and earthworm impact as seedlings establish and mature. It follows the growth, survival and reproduction of a diverse array of native plants over four to six years.

Chapter 5 focuses on the conservation, management and restoration lessons learned from this research.

WORKS CITED

Bohlen PJ, Pelletier DM, Groffman PM, Fahey TJ, Fisk MC. 2004. Influence of earthworm invasion on redistribution and retention of soil carbon and nitrogen in northern temperate forests. Ecosystems 7:13–27.

Côté B, Halloran IO, Hendershot WH, Spankie H. 1995. Possible interference of fertilization in the natural recovery of a declining sugar maple stand in southern Quebec. :471–80.

Dávalos A, Nuzzo V, Blossey B. 2014. Demographic responses of rare forest plants to multiple stressors: The role of deer, invasive species and nutrients. J Ecol 102:1222–33.

Didham RK, Tylianakis JM, Hutchison MA, Ewers RM, Gemmell NJ. 2005. Are invasive species the drivers of ecological change? Trends Ecol Evol 20:470–4.

Dobson A, Blossey B. 2015. Earthworm invasion, white-tailed deer and seedling establishment in deciduous forests of north-eastern North America. J Ecol 103:153–64.

Eisenhauer N, Fisichelli NA, Frelich LE, Reich PB. 2012. Interactive effects of global warming and 'global worming' on the initial establishment of native and exotic herbaceous plant species. Oikos 121:1121–33.

Frelich LE, Hale CM, Scheu S, Holdsworth AR, Heneghan L, Bohlen PJ, Reich PB. 2006. Earthworm invasion into previously earthworm-free temperate and boreal forests. Biol Invasions 8:1235–45.

Gilliam FS. 2007. The ecological significance of the herbaceous layer in temperate forest ecosystems. Bioscience 57:845.

Gilliam FS. 2014. The herbaceous layer in forests of eastern North America. 2nd ed. (Gilliam FS, editor.). New York, New York, USA: Oxford University Press

Hale CM, Frelich LE, Reich PB. 2006. Changes in hardwood forest understory plant communities in response to European earthworm invasions. Ecology 87:1637–49.

Hale CM, Frelich LE, Reich PB, Pastor J. 2008. Exotic earthworm effects on hardwood forest floor, nutrient availability and native plants: A mesocosm study. Oecologia 155:509–18.

Holdsworth AR, Frelich LE, Reich PB. 2007. Effects of earthworm invasion on plant species richness in northern hardwood forests. Conserv Biol 21:997–1008.

Larson ER, Kipfmueller KF, Hale CM, Frelich LE, Reich PB. 2010. Tree rings detect earthworm invasions and their effects in northern Hardwood forests. Biol Invasions 12:1053–66.

Leonard JA, Field JB. 2004. Distributions of cations in the regolith and vegetation. In: Roach I, editor. Regiolith: Proceedings of the CRC LEME Regional Regolith Symposia 2004. Bentley, WA, Australia: CRC LEME. pp 215–9.

MacDougall AS, Turkington R. 2005. Are invasive species the drivers or passengers of change in degraded ecosystems? Ecology 86:42–55.

McKinney ML, Lockwood JL. 1999. Biotic homogenization: A few winners replacing many losers in the next mass extinction. Trends Ecol Evol 14:450–3.

Roberts M, Gilliam FS. 2014. Response of the herbaceous layer to disturbance in eastern forests. In: Gilliam FS, editor. The herbaceous layer in forests of eastern North America. 2nd ed. New York: Oxford University Press. pp 321–39.

Suarez ER, Pelletier DM, Fahey TJ, Groffman PM, Bohlen PJ, Fisk MC. 2004. Effects of exotic earthworms on soil phosphorus cycling in two broadleaf temperate forests. Ecosystems 7:28–44.

Wiegmann SM, Waller DM. 2006. Fifty years of change in northern upland forest understories: Identity and traits of ‘winner’ and ‘loser’ plant species. Biol Conserv 129:109–23.

Chapter 2

INVASIVE EARTHWORMS CHANGE NUTRIENT AVAILABILITY AND UPTAKE BY FOREST UNDERSTORY PLANTS [1]

ABSTRACT

Background and Aims Assess whether invasive earthworms alter nutrient dynamics in habitats they colonize.

Methods We investigated nutrient dynamics of forest soils and three native plant species (*Acer saccharum, Polygonatum pubescens, Polystichum acrostichoides*) along four earthworm invasion gradients in central New York.

Results Earthworm biomass (a proxy for earthworm impact) was related to distribution and concentration of soil and plant nutrients. At shallower depths, earthworms were associated with lower total and extractable P, but higher Ca, K, Mg and Mn. Earthworm-invaded plots showed higher soil Ca and higher foliar Ca in *A. saccharum* and *P. acrostichoides*, and lower soil P with lower foliar P in *P. pubescens*. Presence of earthworms substantially decreased rooting volume in the A horizon, co-occurring with a build up of extractable nutrient concentrations and pools.

Conclusions Overall, earthworm biomass was a better predictor of foliar nutrient concentrations than either extractable or total nutrient concentrations and pools. Earthworms may create stressful rooting conditions, limiting rooting of native plants in the A horizon. The resulting plant-accessible nutrient pool that builds up in the A horizon of earthworm-invaded soils could provide a mechanism for the invasive success of non-indigenous plants that have an evolutionary association with earthworms in the native range and that follow earthworm invasions.

[1] Dobson AM, Blossey B, Richardson JB. (2017). Plant and Soil. Invasive earthworms change nutrient availability and uptake by forest understory plants. 421(1-2): 175-190. Used here with permission from Springer Nature, license number 4253661281508.

INTRODUCTION

Historically, north-eastern North American forests represent nutrient-limited systems with occasional temporal and spatial nutrient flushes (Gilliam 2014). As a result, native plants have evolved unique strategies such as complex mycorrhizal associations, gender shifts and extended dormancy to maintain optimal stoichiometry across a range of soil fertility (Doust, Jon L. Cavers 1982; Boerner 1986a; Kery and Gregg 2004). Following the last glacial maximum approximately 22,000 yrs ago, north-eastern North American forests have developed in the absence of earthworms (James 1995; Hendrix and Bohlen 2002). These forest soils are often starkly stratified, and organic inputs build up to create a well-developed organic horizon (Bohlen et al. 2004c; Frelich et al. 2006; Hale et al. 2008).

As a result, macro (such as Ca, N, P, K, S) and mesonutrient (Mg, Mn) cycles are tightly coupled with forest plants, mycorrhizal symbionts, and microorganisms to maximize nutrient retention in shallow horizons through close coupling of decomposition and uptake processes (Leonard and Field 2004; Suarez et al. 2004; Gilliam 2014). P cycling is particularly tightly regulated due to its commonly limiting concentration in acidic forest soils, and little P is leached from the A horizon (Wood et al. 1984; Yanai 1992; Gilliam 2014). Calcium, Mg, K and S availability have varying degrees of limitation in many forests ecosystems in the region, and acid rain has caused a substantial decrease in extractable Ca in forest soils (Huntington et al. 2000; Kobe et al. 2002; Jenkins et al. 2005). These nutrients serve many physiological functions, and are crucial for biological processes such as photosynthesis, chlorophyll production, chemical signaling, root foraging, or mycorrhizal colonization (Ellsworth and Liu 1994; Long et al. 1997; Horsley et al. 2000; Kobe et al. 2002; Clair and Lynch 2005; Juice et al. 2006). In addition to physiological problems of Ca, Mg and K deficiencies, soils may lose their pH buffering capacity,

and Al and Mn toxicity can become problematic (Elliott 2009). Calcium losses limit photosynthetic capacity, contributing to issues such as canopy dieback, and is so severe that growth simulations predict that Ca fertilization of 10 g m^{-2}led to more than a doubling of *A. saccharum* basal area in forests in one tree generation (Ellsworth and Liu 1994; Kobe et al. 2002).

In Appalachian forests, extractable Mn is associated with increased understory plant diversity (Newell and Peet 1998). Plants preferentially take up Ca over Mn when it is available, but acid deposition and subsequent Ca deficiency (as well as high Mn availability) in soils can lead to Mn toxicity (Juice et al. 2006). In acidic forests, higher Mn (and lower Mg) foliar concentrations are associated with sugar maple decline (Horsley et al. 2000). Tree species display a breadth of susceptibility to Mn toxicity, suggesting soil Mn could be an important driver of forest species assemblages (St. Clair and Lynch 2005).

A myriad of anthropogenic influences alter nutrient cycling, which threatens this closely coupled system (Côté et al. 1995). To conserve species, ecosystems, and diversity of forests, it is vitally important that we understand the consequences of novel perturbations to nutrient dynamics. The understory has received relatively less consideration in forest ecology, but acts as a filter of future species pools by determining which seeds and propagules can establish (Dorning and Cipollini 2006; Royo and Carson 2008; Gilliam 2014). It is diverse (up to 90% of plant biodiversity), and is the interface of below and above-ground interactions (Gilliam 2014).

Though low in aboveground biomass (<1 %) relative to canopy trees, herbaceous understory plants are important components of nutrient cycling (Gilliam 2014). Litter from understory plants is typically high quality, and can contribute up to 20 - 25 % of litterfall in a season (Gilliam 2014). Understanding nutrient dynamics is important to project future species

assemblages. For example, decreased nutrient availability has been strongly linked with sugar maple decline, a socially and economically important species (Kolb and McCormick 1993; Horsley et al. 2002). Additionally, disturbances can promote invasive plants or early successional species that are better able to take advantage of elevated labile macronutrient concentrations even in the absence of disturbance (Huenneke et al. 1990; Schaberg et al. 2006; Szlavecz et al. 2011).

European and more recently Asian earthworms have been introduced by human activities (Scheu and Parkinson 1994; Burtelow et al. 1998; Bohlen et al. 2004c). As soil engineers, the influence of earthworm invasion propagates throughout entire ecosystems. In forests, the most noticeable change is rapid decomposition and redistribution of organic matter, often leading to a complete loss of the O horizon (Bohlen et al. 2004c; Hale et al. 2008). The outcome of earthworm invasion on individual soil nutrients is inconsistent, and depends on soil type, earthworm species assemblages, time since invasion and existing soil community (Bohlen et al. 2004a; Suarez et al. 2006a; Filley et al. 2008). Despite this complexity, earthworm-associated changes to C and N cycling have been well described, although other important forest nutrients have received considerably less attention (Scheu 1994; Bityutskii et al. 2002; Bohlen et al. 2004b; Wironen and Moore 2006; Weihua and Xiuqin 2007; Umarov et al. 2008). Resner et al. (2015) observed an initial enrichment of Ca, K, Mg and P in the A-horizon along an earthworm invasion gradient, but an overall depletion of these nutrients in the soils with the longest earthworm invasion history. Availability of nutrients to native understory plants may decline with increased leaching out of the rooting zone (Bohlen et al. 2004a; Hale et al. 2005; Bal et al. 2017).

The effect of earthworms on P cycling is particularly complex, and is dependent on soil type, nutrient fraction, earthworm species, and time since invasion (Bohlen et al. 2004c; Suarez et al. 2004). Previous studies in tropical forests have observed earthworms increase total and plant available P (De Vleeschauwer and Lal 1981; Zhang et al. 2000; Kuczak et al. 2006; Chapuis-Lardy et al. 2011). In temperate agricultural systems earthworms have been associated with a decrease in inaccessible organic P, but increased water-soluble inorganic P (Coulis et al. 2014). In central New York forests, Suarez et al. (2004) found earthworms influence on different P fractions to be variable in direction and scale. In a mesocosm study, Hale et al. (2008) demonstrated that diverse earthworm communities increased available phosphates, although individual earthworm species had no effect. Taken together, this could suggest initial increases in organic P mineralization in early stages of earthworm invasions may be followed by a decrease in available P due to leaching (Bohlen et al. 2004c).

Earthworm influences on soil nutrient dynamics will determine understory plant assemblages, as some species are sensitive to changes while others may thrive. Including plants in the conceptual model of impacts of earthworm invasion on nutrient cycling is the next step to understanding their fundamental impact to forest taxa. We investigated *A. saccharum*, *Polygonatum pubescens* (Willd.) Pursh and *Polystichum acrostichoides* (Michx.) Schott, three important understory species with contrasting life histories and variable survival and growth responses to earthworm invasion. By including a tree, an herbaceous perennial and a fern, we were able to understand whether the stoichiometry of plants with different growth strategies vary in response to earthworm invasion. While an understanding of precisely what causes a plant to be vulnerable to earthworm invasion is still limited, we chose study plants that vary in traits that may be associated with increased sensitivity to earthworm invasion such as high mycorrhizal

dependence and specificity, shallow rooting architecture and roots lacking chemical and physical protection from herbivory (Gundale 2002; Lawrence et al. 2003; Hale et al. 2006a; McLean et al. 2006; Drouin et al. 2016).

In previous experiments, *P. acrostichoides* benefited from earthworms (Bowe et al., unpublished), while *P. pubescens* survival was negatively affected by earthworms (Dobson and Blossey 2015). Negative relationships between invasive earthworms and *A. saccharum*, have been identified through field and mesocosm studies in the Midwest (Holdsworth et al. 2007a; Hale et al. 2008; Corio et al. 2009; Bal et al. 2017). *Polystichum acrostichoides* is an evergreen fern with branched roots growing from a perennial rhizome, variable (and possibly facultative) degrees of mycorrhizal colonization, and broad mycorrhizal compatibility (Berch and Kendrick 1982; West et al. 2009). The root biomass of *P. pubescens* consists of a large storage rhizome with a few small, largely unbranched roots, and arbuscular mycorrhizal colonization ranges from moderate to very high (Boerner 1986b; Brundrett and Kendrick 1988; Dobson, unpublished). Roots of *A. saccharum* are highly chemically and physically protected, and have moderate levels of arbuscular mycorrhizal colonization (Brundrett and Kendrick 1988).

Objectives

The objective of this study was to quantify macro and mesonutrients in soils and plants to assess the potential impacts on understory vegetation by earthworm invasions. We tested several related hypotheses: (1) earthworm presence decreases total and extractable soil nutrients; (2) earthworms mix soil horizons, causing vertical homogenization of soil nutrient concentrations; and (3) earthworm-associated changes in extractable soil nutrients will trigger analogous changes in nutrient concentration of plant foliage.

METHODS

Study area

We selected four forests (Bobolink Hill, Hammond Hill State Forest, Ringwood Preserve and Yellow Barn State Forest) in Tompkins and Tioga counties in the Finger Lakes Region of New York State (Appendix I.7). All forests fall within the Allegheny section of the Appalachian Plateau at approximately 42°N, 76°W with acidic (pH 3.9 – 5.0) Fragiaquepts and Dystrochrepts soil in the Mardin and Volusia series (SoilWeb, USDA-NRCS & UC Davis California 2010). Mixed hardwood canopies are predominantly closed (Leaf Area Index 5-7), and dominated by *A. saccharu*m, *Fraxinus* spp., *Fagus grandifolia* Ehrh. and *Quercus rubra* L.

We selected six plots in each forest along a 0.5 – 2 km gradient from earthworm-free to earthworm-invaded areas. Land use history varied among forests, however all plots within a forest had similar land-use history (Appendix I.7). Earthworm-invasion histories are unknown, however all plots at Ringwood, Yellow Barn and Bobolink Hill retained traces (<5 cm depth) of an organic horizon. Each plot contained all three study species: *A. saccharum*, *P. pubescens*, and *P. acrostichoides* within a 15m diameter sampling area. Despite their different responses to earthworms, all plant species were present at all plots with and without earthworm invasions, with the exception of *A. saccharum* in the uninvaded plot at Yellow Barn State Forest.

Plant sampling

In July 2016, we collected *A. saccharum* saplings (15–50 cm), non-reproductive *P. pubescens* and reproductive *P. acrostichoides* individuals in each plot, taking care to excavate the entire root mass. We rejected any plants showing signs of senescence, insect or pathogen damage. To ensure sufficient foliar material for nutrient analyses, we collected one to three *A. saccharum* saplings and *P. pubescens* stalks in each plot. We triple-rinsed and removed all visible soil from

roots, and air dried samples in a greenhouse for one week. We separated leaves (removing petioles and stems) and roots, and ground each sample using a coffee grinder (Fresh Grind, E160BY, Proctor Silex, Southern Pines, NC, USA). For *A. saccharum*, we separated and discarded the primary root from actively growing roots. We used roots, but not rhizomes of *P. acrostichoides*, but due to low biomass of secondary roots, we ground entire *P. pubescens* root masses.

Earthworm sampling

All plots were chosen from within large, long term sampling sites (Dobson and Blossey 2015). Therefore, we were able to use earthworm biomass data from 10 locations along two diagonal 50 m transects around each plant sampling plot in autumn 2012, 2013 and 2015 using liquid mustard extraction (3 g powdered mustard 3.79 L^{-1} water poured into a 0.5 m^{-1} sampling frame) (Lawrence and Bowers 2002). We collected all surfacing earthworms and preserved them in formalin for 48 h before transferring them into ethanol for storage. We identified all adult individuals to species and juveniles to genus, and classified them to ecophysiological group, including epigeic (litter-dwellers), endogeic (soil-dwellers), anecic (deep burrowing surface dwellers), and epi-endogeic (soil/litter dwellers) (Edwards and Bohlen 1996; Deleporte 2001; Hale et al. 2005). We then pooled all 10 samples from within each plot and dried them at 60 °C for 72 h to determine dry biomass. We used the 3-year mean of this pooled plot-level earthworm biomass for analyses to minimize influence of annual variability and microhabitat heterogeneity.

Soil sampling

At each plot, we excavated one soil monolith at a random location near the approximate plot center. To avoid direct effects from mature trees, we excavated monoliths at least 1 m away from any trees. A 15 cm^2 template was randomly placed on the soil surface. We cut O horizons using a stainless steel handsaw and soil surrounding the template was removed to expose the soil monolith. We noted horizon depths and soil morphological features for each soil monolith. We then separated the soil monolith by master horizons and collected each entire horizon. In the laboratory, we air dried each master horizon (O horizon, A horizon, and B horizon) and sieved to < 2 mm. Only two earthworm-invaded pits had sufficient O horizon mass to analyze, thus we could not include it in further evaluations. We quantified rock mass (> 2 mm) and root mass (> 1 mm) for each soil horizon. We calculated bulk density by dividing air dried, sieved soil mass for each horizon by horizon depth measured in the field.

Plant and soil analyses

We included plot-level pH measurements recorded in summer 2014 (Dobson and Blossey 2015). To measure % soil organic matter (SOM), we dried horizons separately at 60°C for 48 h, followed by grinding (DynaCrush; Customer Laboratory Inc., Orange City, FL, USA) sieving (1.18 mm) and igniting at 360°C for 2 h in a muffle furnace. This temperature combusts organic material, while maintaining inorganic soil constituents.

To characterize extractable/weakly bound metal fractions, we extracted 2 g of soil with 20 mL of 1 M ammonium acetate (Chapman 1965; Ciesielski et al. 1997). Samples were shaken for 1 hr and allowed to equilibrate for 24 hrs. We then centrifuged soil slurries at 3000 rpm for 30 min and the extraction solution was decanted. A pseudo-total digestion was used to measure total nutrient concentrations in plant tissues and soil samples following EPA method 3051A.

This method is often referred to as a pseudo-total digestion due to the inability to dissolve silicates and other refractory compounds. These refractory compounds are dominant in soils but may also be present in plants. Since our study focuses on plant available nutrients, we henceforth refer to the pseudo-total concentrations as total concentrations. In the digestion method, 0.500 grams of air-dried material was digested in 5 ml of 8 M reverse aqua regia (9:1, HNO_3 : HCl). Materials were digested at 90°C for 45 min on an insulated hot plate in sealed teflon vials. We further diluted digestate and analyzed via ICP-OES (SPECTRO Analytical Instruments, Kleve, Germany). Every 25 samples included a digestion blank, a duplicate and 2 standard reference materials (SRM). We used matching sample matrices from the National Institute of Standards and Technology: Montana Soil 2711 for mineral soil samples and Peach Leaves 1547 for plant tissue samples (National Institute of Standards and Technology, Gaithersburg, MD). Relative standard deviations (RSDs) among SRMs and duplicate samples were less than < 4 % for all total concentrations in plants and soils and < 8 % for extractable soil concentrations. Preparation blanks had elemental concentrations below detection limit for Ca, Mg, Mn, P and S. Potassium concentrations within blanks were < 4 $\mu g\ L^{-1}$, or roughly a 0.2 % of sample concentrations. As expected, total concentration recoveries for Montana Soil SRM were between 60 – 90 % for all nutrients particularly due to the indigestion of silicate and other residual compounds. Total concentration recoveries for Peach Leaves SRM were Ca (77 %), Mg (87 %), K (103 %), Mn (76 %), P (72 %), and S (65 %). Similar to soil extractions, recoveries were < 100 % for many elements due to residual compounds within plant tissues.

Data analyses

We tested the influence of earthworm activity on three response variables: (1) soil horizon nutrient concentration, (2) 0 – 10 and 10 – 20 cm nutrient pools, and (3) plant nutrient concentration using linear mixed models (LMMs) (package lme4). Each set of models included dry earthworm biomass as a fixed effect, with site as a random effect. We analyzed nutrient concentrations as separate response variables for each nutrient and each horizon. Each pit had an A horizon (n = 24), however the sample size for B horizons (n = 18) is depreciated because pits from heavily invaded soils contained one deep, homogenous A horizon, consistent with earthworm mixing. Since A horizon and B horizon depths varied significantly among plots and strongly influenced total volume of soil horizons, we calculated nutrient pools using standardized depths of 10 cm and 10-20 cm, henceforth referred to as normalized A horizon and normalized B horizon, respectively. We adjusted normalized A horizon and B horizon pools to 10 cm intervals using their bulk density. Due to orders of magnitude difference in nutrient content among pits, we log-transformed normalized horizons for analysis with LMMs. For plant nutrient models, we analyzed root and leaf tissue for each species separately. In addition to LMMs, we qualitatively compared foliar seedling nutrient concentrations to the minimum published concentration for healthy July *A. saccharum* foliage following Burton et al. (1993), Kolb, T.E.; McCormick (1993) and Ca : Al toxicity from Cronan and Grigal (1995) to infer whether different patterns emerged above and below these thresholds.

Next, we tested whether earthworms altered plant-soil relations, again using LMMs. We evaluated concentrations of each nutrient (response variables) of each plant species with independent models, with site as a random effect. We used the interaction between earthworm presence/absence or earthworm biomass and nutrient pools from the top 10 cm of soil (to reflect rooting depth of our plants) as fixed effects. Using root biomass as a response variable, we

coarsely modeled vertical distribution of roots in our soil pits using the interaction between earthworm biomass and horizon (A and B only) as fixed effects, with site as a random effect.

Finally, we tested the relationship between earthworms biomass (fixed effect) and cation exchange capacity (CEC), pH and % soil organic matter (SOM) (independent response variables), with site as a random effect. We tested CEC, and pH of the top 10 cm only, but ran separate models for SOM in each horizon. We performed all analyses using R statistical software (R Core Team 2014).

RESULTS

Earthworm sampling

Lumbricus terrestris L. and *Lumbricus rubellus* Hoffmeister dominated earthworm populations at all sites, while endogeic species in the *Aporrectodea calignosa* complex (*Aporrectodea tuberculata* Eisen, *Aporrectodea calignosa* Savigny, *Aporrectodea trapezoids* Dugés) were co-dominant with *Lumbricus* sp. at Hammond Hill and Bobolink Hill (Appendix I.1). At all sites except Ringwood, we observed moderate abundances of *Octolasion tyrtaeum* Savigny, *Octolasion cyaneum* Savigny, *Aporrectodea rosea* Savigny and *Dendrobaena octaedra* Savigny and *Dendrodrillus rubidus* Savigny (Appendix I.1). Within sites, low-earthworm biomass plots (0.004 – 0.64 g m^{-2}, 0 – 10 individuals m^2) included *D. octaedra*, *D. rubidus* and *L. rubellus*. Moderately invaded sites (1.76 – 2.16 g m^{-2}, 29 - 43 individuals m^2) were dominated by *Lumbricus* sp., and *Octolasion* sp. and *D. octaedra,* and heavily-invaded plots (3.27 – 3.63 g m^{-2}, 21 – 63 individuals m^2) maintained a diverse assemblage of species from all ecophysiological groups (Appendix I.1 & I.3).

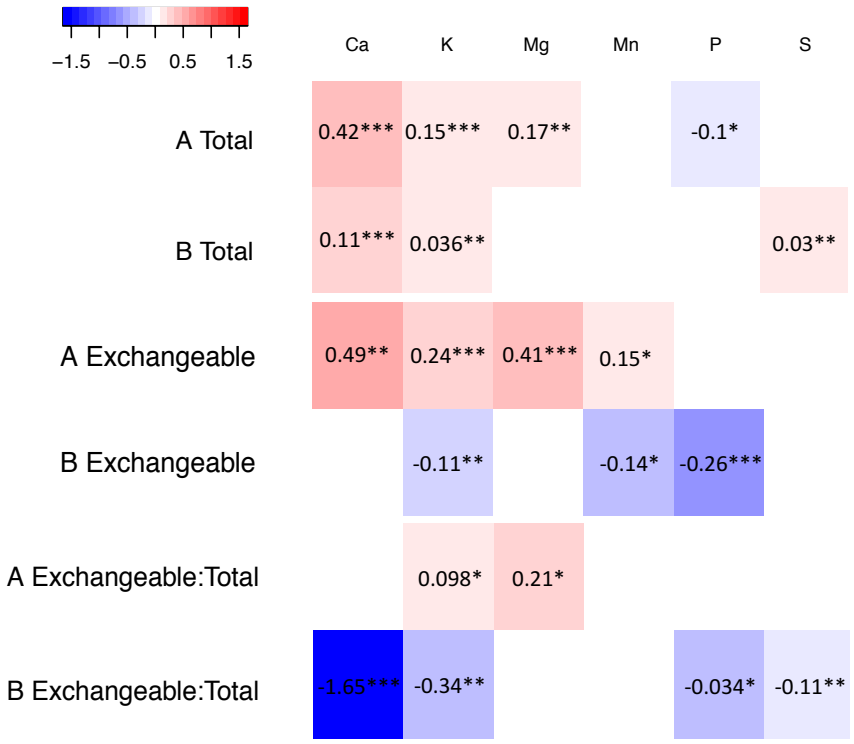


Figure 2-1. Effect of earthworm biomass on total, extractable and extractable:total soil nutrient concentration (mg kg-1). Colored boxes represent parameter estimates of earthworm biomass from mixed models fitted with maximum likelihood. Earthworm biomass was the sole fixed effect and site is the random effect. To compare across nutrients, raw response variables were standardized by dividing by their mean. Red and blue represent direction of the relationship (positive and negative, respectively), and saturation represents the parameter itself. Raw plots in Appendices I.2 & I.3, summary statistics are in Appendix I.8. P-values are estimated from a normal distribution (* P < .05; ** P < .01, *** P < .001).

Earthworms and soil

Consequences of earthworm invasion were variable for nutrient concentrations and pools in the A and B horizons. In the A horizon, higher earthworm biomass was associated with higher concentrations of total Ca, K, and Mg and extractable Ca, K, Mg, and Mn but lower total P (Fig. 2-1; Appendices I.2 & I.3; Appendix I.8). In contrast, extractable K, Mn, and P in the B horizon were depleted with increasing earthworm biomass, although higher total Ca and K was maintained in addition to higher S (Fig. 2-1; Appendices I.2 & I.3; Appendix I.8). Earthworm

biomass was also associated with lower ratios of extractable:total Ca, K, P and S in the B horizon (Fig. 2-1; Appendices I.2 & I.3; Appendix I.8). In the A horizon, earthworm biomass was associated with higher extractable:total concentrations of Mg and Mn, and small but significantly higher S concentrations (Fig. 2-1; Appendices I.2 & I.3; Appendix I.8). We observed a negative effect of earthworm biomass on P at 10-20 cm depth, but also observed a positive effect on total Ca and extractable Mg (Fig. 2-3; Appendix I.9). Nutrient pools were consistent with concentration patterns within the A horizon, with greater earthworm biomass predicting higher Ca, K, Mg and extractable Mn in the top 10 cm (Fig. 2-2; Appendix I.9).

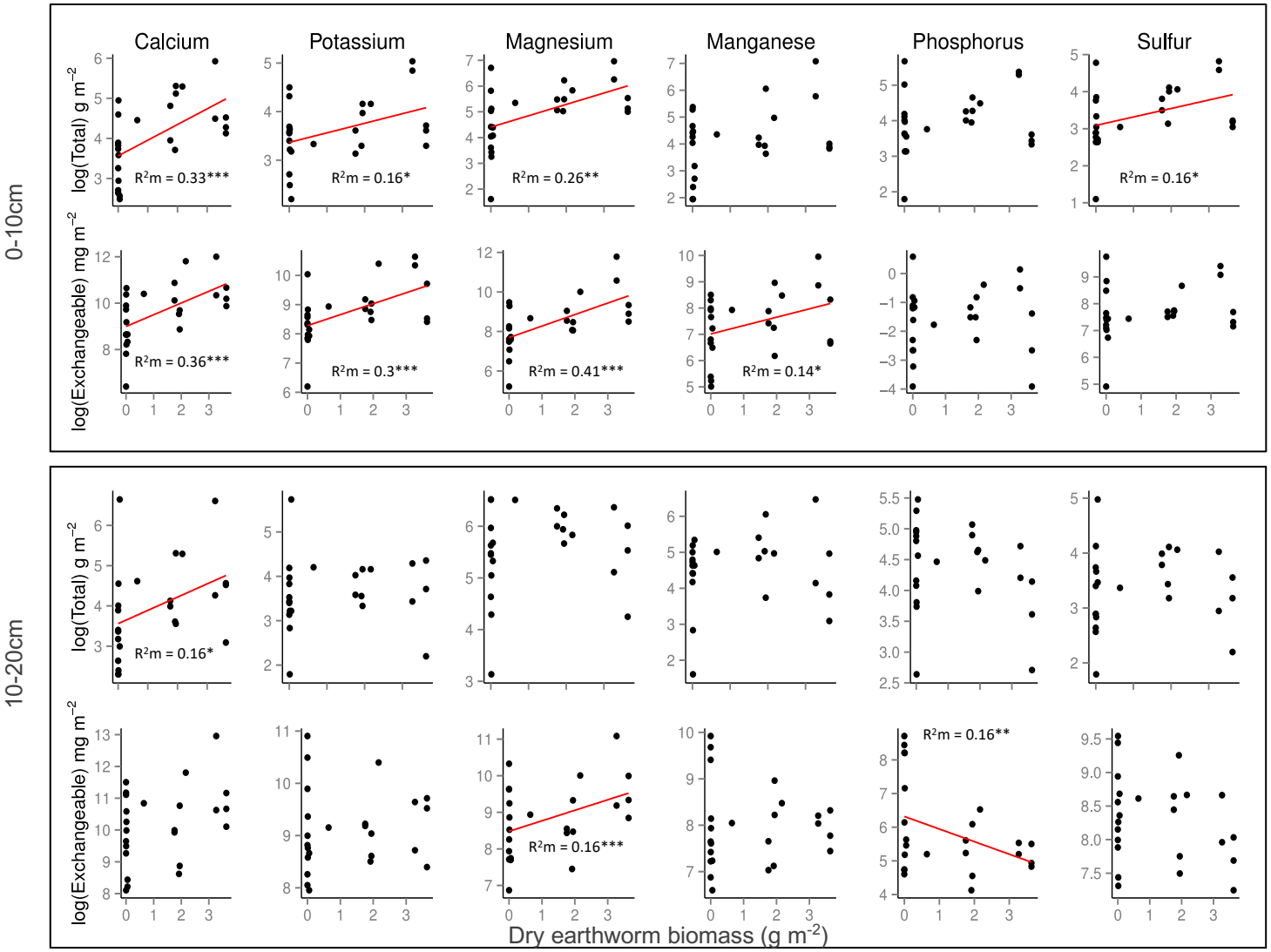


Figure 2-2. Elemental total (g m-2) and extractable nutrient pools (g m-2) in shallow (0 - 10 cm soil depth; top panel) and deep (10 - 20 cm soil depth; lower panel) as a function of dry earthworm biomass (g m-2). Data are log-transformed standardized (N = 24 plots). Panel with lines represent significant linear mixed model predictions. Summary statistics are provided in Appendix I.9. P-values are estimated from a normal distribution (* P < .05; ** P < .01, *** P < .001).

Earthworm influence on plant nutrition

With exception of *P. pubescens*, increased earthworm biomass predicted higher concentrations of foliar (Fig. 2-3; Appendix I.10) and root (Fig. 2-4; Appendix I.10) Ca and lower P concentrations in all roots, and *P. pubescens* leaves. Despite having a positive effect on extractable soil Mn in the A horizon (Figs 1 & 2), both foliar and root Mn concentrations declined with increasing earthworm biomass for all species (Figs 3 & 4; Appendix I.5; Appendix I.10). Further, higher soil extractable Mg, and K in earthworm-invaded plots did not translate into higher tissue Mg or K concentrations (Figs 3 & 4; Appendix I.5; Appendix I.10).

Soil nutrient concentration and pools were highly variable and were poor predictors of plant tissue concentrations. Further, we did not find evidence for earthworm x soil nutrient interactions for *P. pubescens* or *P. acrostichoides*. We found a positive relationship between soil and *A. saccharum* tissue Ca in non-earthworm-invaded plots (Appendix I.6; Appendix I.11). Surprisingly, for most other species and many other nutrients, earthworm biomass alone was a much better predictor of plant tissue nutritional concentrations (Fig. 2-3; Appendix I.10).

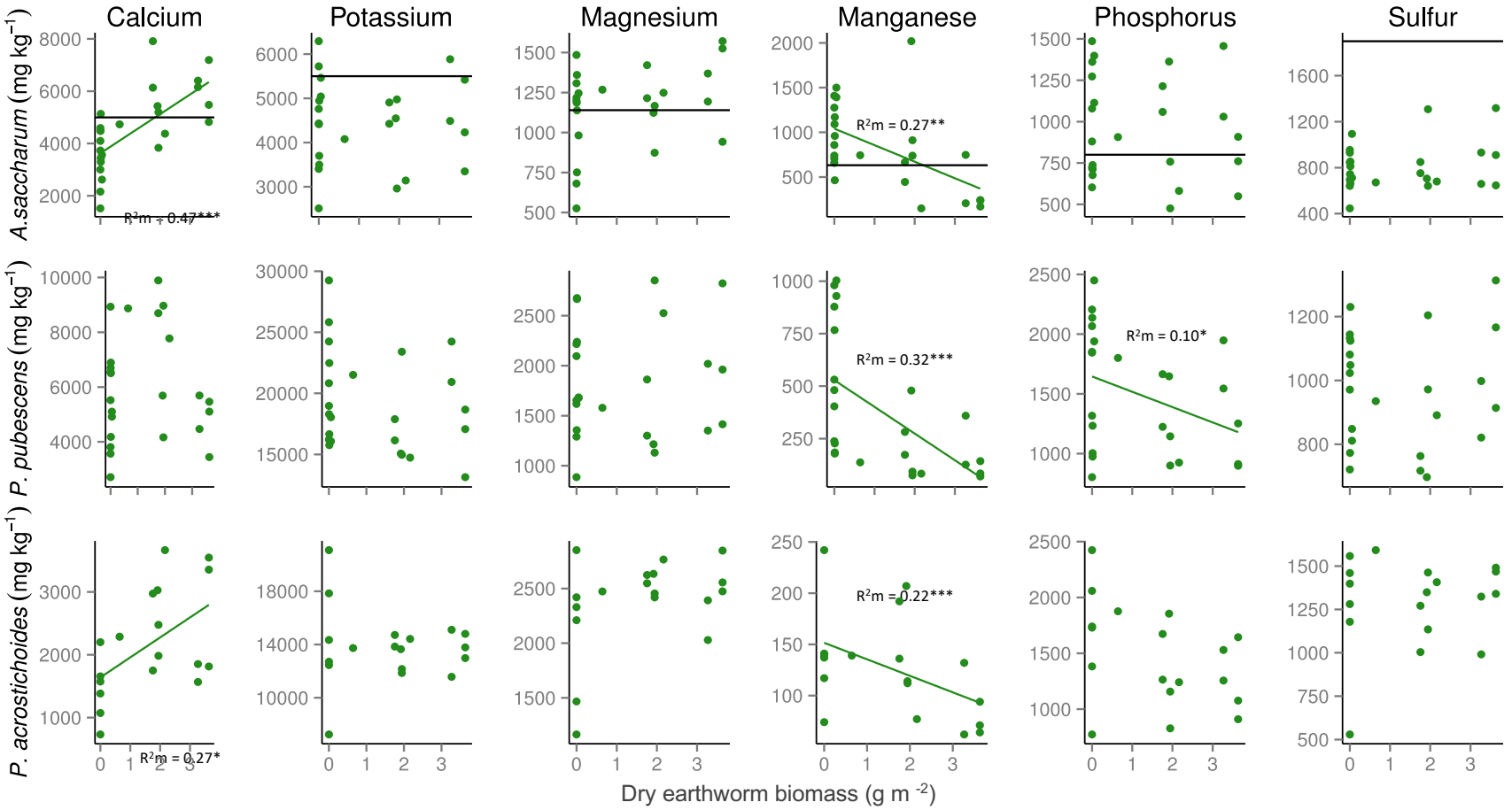


Figure 2-3. Relationship between plant leaf tissue (mg kg-1) and earthworm biomass (g m-2). Horizontal line represents minimum published range for healthy Acer saccharum trees grown in unfertilized forest conditions in July/August (Burton et al. 1993; Kolb and McCormack).

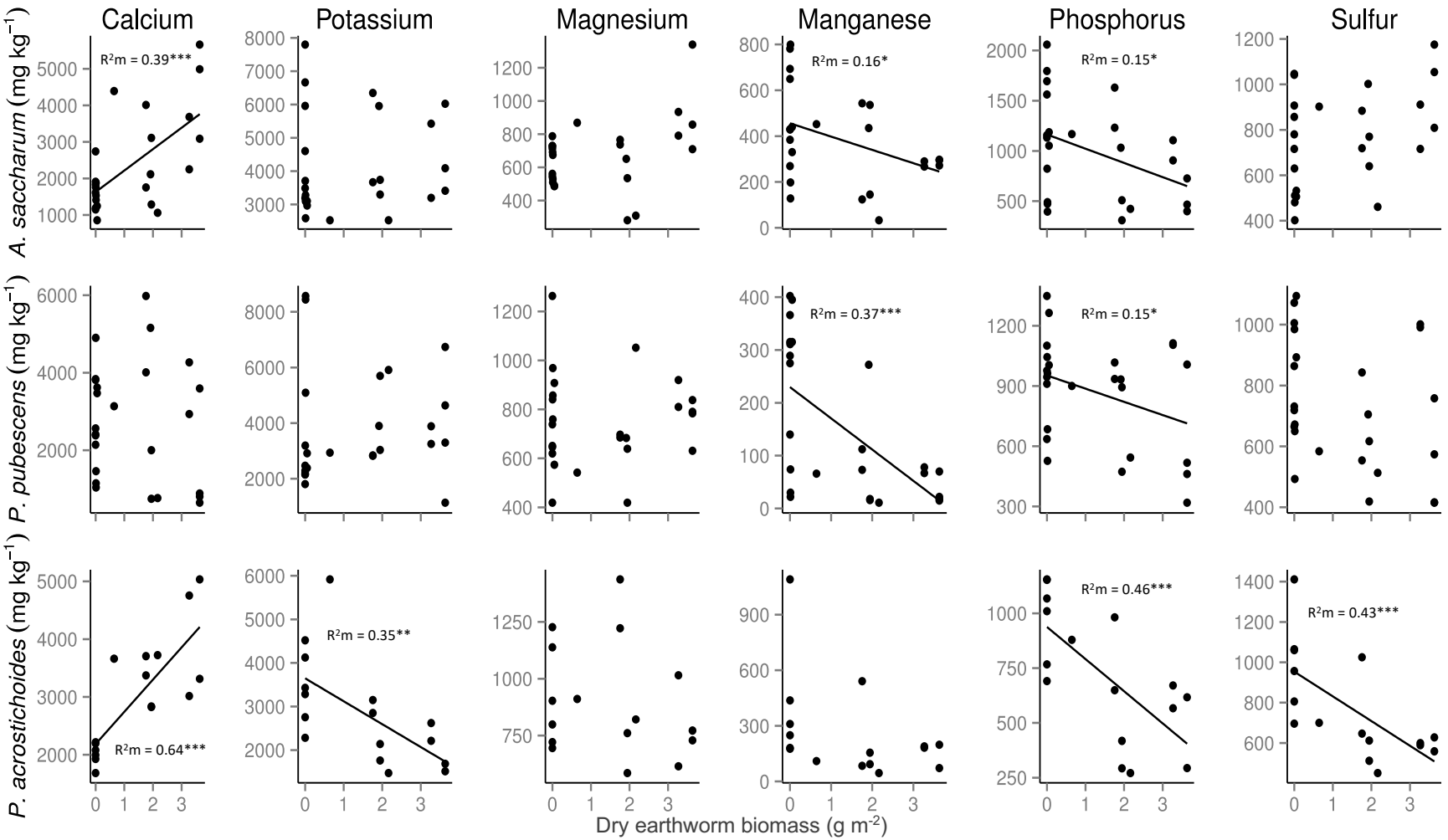


Figure 2-4. . Relationship between plant root tissue (mg kg-1) and earthworm biomass (g m-2). Summary statistics are provided in Appendix I.10. P-values are estimated from a normal distribution (* P < .05; ** P < .01, *** P < .001).

Earthworm influence on other soil conditions

Earthworm biomass had a positive, though nonsignificant relationship with CEC ($R^2_m = 0.14$, $p = 0.057$). However, this was driven by a single datum with a CEC one order of magnitude larger than the rest, and the trend remained nonsignificant when the single datum is removed from analysis ($R^2_m = 0.08$, $p = 0.14$). Earthworm biomass was positively associated with soil pH ($R^2_m = 0.44$, $p < 0.001$), but did not correspond with any trends with SOM for any horizon. Interestingly, we found a significant interaction between earthworm presence and horizon in our pits, with roots preferentially growing in the B horizon in earthworm-invaded conditions and in the A horizon in the absence of earthworms (Fig. 2-5; Appendix I.12). However, using continuous earthworm biomass data, the earthworm biomass*horizon interaction was not significant predictor of root biomass.

DISCUSSION

Our results support a growing literature suggesting earthworm-invaded forests have conspicuously different nutrient dynamics than uninvaded forests. On the basis of previous studies of earthworm impact on N cycling, we hypothesized that exotic earthworms would negatively impact extractable and total macro and mesonutrients soil concentrations, but observed that the effect varied among nutrients and between soil horizons (Bohlen et al. 2004b; Wironen and Moore 2006; Szlavecz et al. 2006; Eisenhauer et al. 2007). Earthworm-invaded plots were characterized by higher total Ca, K and Mg and extractable Ca, K, Mg and Mn, particularly for A horizons (Figs. 2-1, 2-2 & 2-6). This is likely due to mineralization of the O horizon and incorporation of those nutrients in the A horizon, which is consistent with patterns of early epi-endogeic earthworm invasions observed by previous studies such as Resner et al. (2015) in Midwestern hardwood stands. Although we do not have information about the time since earthworm invasion, plots at three of four sites retained

traces of an organic horizon, either because the invasion is relatively new or the habitat is only conducive to low earthworm densities.

Assuming Ca and Mg concentrations at uninvaded plots are estimates of baseline, we approximate that earthworms have increased Ca and Mg concentrations and pools 75 – 526 % (Figs. 2-1, 2-2). It is possible that elevated Ca and Mg occurred due to earthworms affecting the chemical nature of SOM. For example, Knowles et al. (2016) found *A. tuberculata* altered the physicochemical properties of soil C and restructured SOM. These physicochemical changes to SOM can directly impact SOM sorption capacity and exchangeability for cations like Ca and Mg (Reich et al. 2005). However, we did not observe a significant relationship between earthworm biomass and cation exchange capacity nor SOM (Appendix I.6). Thus, it is unlikely that differences in extractable or total nutrient concentrations were caused by earthworms affecting the sorption capacity of SOM. An additional hypothesis is that earthworm distributions may follow nutrient heterogeneity in the landscape. Earthworm preference for high-Ca substrates can be remarkably finely tuned, with earthworm biomass responding to individual trees with Ca-rich litter (Reich et al. 2005), although this may be exclusive to anecic earthworms such as *L. terrestris* (Schelfhout et al. 2017). Additionally, earthworm biomass is positively associated with pH, possibly due to higher soil Ca to buffer acidity, which Elliott (2009) proposes supports a more diverse understory community with higher quality litter. With evidence that earthworms, Mg/Ca concentrations, and higher pH co-vary, it is possible that they create a positive base-cation-earthworm feedback loop (Appendix I.6).

Although many of the nutrient metals had higher concentrations in earthworm-invaded soils, total and extractable P declined with increasing earthworm biomass in A and B horizons, respectively (Figs 1, 2, 6). Assuming P concentrations at uninvaded plots are baseline estimates, we estimated that earthworms decreased P concentrations from 2 - 25 %.

Even small declines in P are profoundly important in these forests, where many plants are near or below the P deficiency threshold (Fig. 2-3). Our results suggest earthworms have a negative impact on P, and similar declines in P were found by Paré and Bernier (1989) where forest soils with earthworm-mixed horizons lost extractable P under acidic conditions. Depleted soil P is consistent with later stages of earthworm invasion, due to mineralization and leaching of the O horizon or rapid mobilization into microbial biomass once earthworms have established (Le Bayon and Binet 2006; Resner et al. 2015). The inverse relationship between Ca and P is notable because soil Ca and P often covary (Figs. 2-1, 2-2). For example, Kobe et al. (2002), saw foliar P, Mg and K increase in *A. saccharum* following Ca fertilization, and Boerner (1986), observed a significant positive correlation between natural Ca and phosphate concentrations in soil in oak-maple forests of Ohio. However, this may not be universally true, as Long et al. (1997) found Ca and Mg fertilization decreased extractable soil P. They hypothesized that under fertilization treatments, P formed insoluble Ca phosphates or was taken up by stimulated roots. However, our results suggest that while Ca is retained, P has been lost from the organic and mineral soil horizons at earthworm-invaded plots.

Independent of Ca, the magnitude and direction of invasive earthworm influence on soil P has been conspicuously variable in previous studies (Suárez et al. 2004; Le Bayon and Binet 2006; Coulis et al. 2014; Vos et al. 2014). Therefore, we must consider biotic and abiotic differences between these studies to interpret our data and understand earthworm impact on P cycling. Earthworm ecophysiological groups influence the pattern of soil mixing, and can increase or decrease nutrient availability depending on dominant earthworm species (Suarez et al. 2006b). Sites dominated by anecic *L. terrestris* maintained higher total P as earthworm's added ions from the deep mineral horizon. In contrast, those dominated by epi-endogeic *L. rubellus* had lower total P as mineralized P was leached away from rooting

zones. Although both *Lumbricus* species were represented at all of our sites (Appendix I.4 & I.6), most individuals are juvenile, and cannot be identified to species. Our sites follow the patterns of epi-endogeic dominance, likely because juvenile *L. terrestris* behave more like epigeic or epi-endogeic feeders (Daniel 1990; Edwards 2004; Asshoff et al. 2010). Taken all together, earthworm-associated increases in Ca, Mg, K, and Mn do not support earthworm-associated depletions suggested in hypothesis (1). However, decreases in P suggest that earthworm-invaded plots could be on the threshold of short-term earthworm-derived enrichments giving way to lower fertility as the remaining organic horizon is consumed, as predicted by our first hypothesis. Additional monitoring through time will be required to quantify these long-term changes to nutrient cycling.

We reject our second hypothesis since earthworm presence did not homogenize the vertical nutrient distribution, and earthworm biomass was associated with higher extractable Ca, K, Mg and Mn concentrations and pools in the A horizon, and lower K, Mn and P concentrations in the B horizon (Fig. 2-1; Appendix I.6). Further, earthworms influenced partitioning of nutrients between extractable and total pools, with a higher relative concentration of extractable nutrients in the A horizon, and lower relative concentration in the B horizon. Surprisingly, this appears to be driven by root absorption, and not mixing of SOM or increased sorption capacity (Fig. 2- 5; Appendix I.6). This has important implications for understanding the role of invasive earthworms in selecting for certain plant species over others (Fig. 2-6).

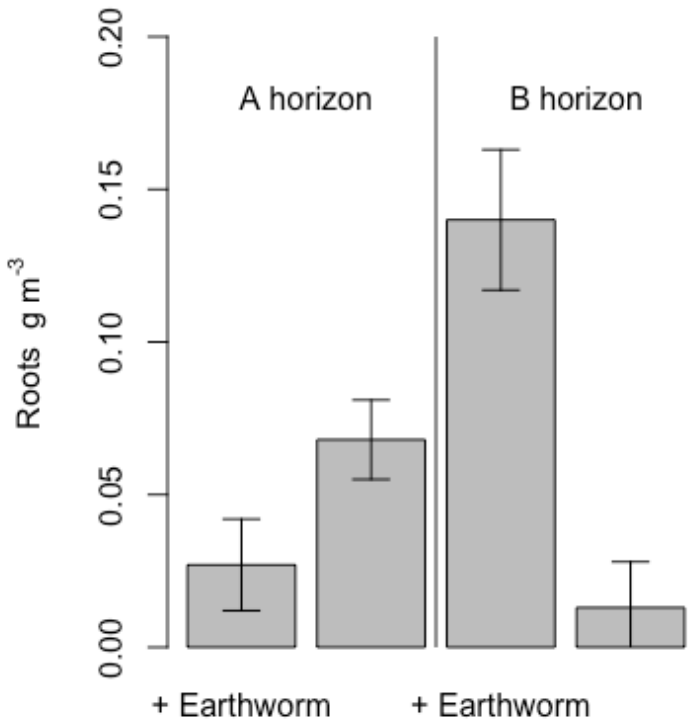


Figure 2-5. Biomass of roots per volume of soil recovered from A and B horizons in earthworm-invaded and uninvaded monoliths (N = 24). Linear mixed models include a significant horizon x earthworm interaction (p = 0.0021). Summary statistics are provided in Appendix I.12.

Earthworms create stressful rooting conditions for many shallow-rooted native plants, requiring those not pre-adapted to these conditions to adapt or perish (Gundale 2002; Bohlen et al. 2004c; Hale et al. 2008). In our earthworm-invaded pits, we observed more rooting in the B horizon, corresponding with depleted extractable nutrient pools (Fig. 2-5). Conversely, in the absence of earthworms, roots predominantly appear in the A horizon (Fig. 2-5). This is somewhat surprising, as one might expect root biomass to correspond to high extractable nutrient concentration (Brady and Weil 2008; Giehl and von Wiren 2014). Direct and indirect stress on shallow-rooted species has been widely suggested as an explanation for the

destructive effect of invasive earthworms on native forest plants, including disruption of mycorrhizal symbioses, decreased soil moisture, production of phytohormones, altered microbial community and root herbivory (Lawrence et al. 2003; Hale et al. 2006b; Holdsworth et al. 2007b; Laossi et al. 2010a; Laossi et al. 2010b; Gilbert et al. 2014; Whitfeld et al. 2014; Dobson and Blossey 2015). Additionally, plants may be rooting deeper for stability where earthworms physically disrupt roots. Because we did not separate roots by diameter, we cannot parse the relative importance of avoiding stressful conditions or a need for greater stability in explaining deeper rooting.

Assuming these deeper roots are not exclusively structural, greater root absorption with greater root biomass could explain lower extractable pools in the B horizon of earthworm-invaded plots . This alternative deep-rooting strategy coupled with depletion in the B horizon could further explain the plant deficiencies of K, Mn, P and S, and lower concentrations of K, Mn and P B horizons of earthworm-invaded soils (Fig. 2-1,3,4,6). In other words, vertical root distribution is inversely related to extractable soil fertility in earthworm-invaded plots, strongly suggesting that large pools of biologically available nutrients build up in the A horizon of earthworm-invaded soils that are inhospitable to roots of native understory plants. This could provide the missing link to explain how earthworms facilitate invasive plants at the expense of some native perennials (Heneghan et al. 2007; Nuzzo et al. 2009).

Invasive plants often thrive with increased soil fertility (Huenneke et al. 1990), and those that have co-evolved with earthworms may have germination and rooting strategies conducive to earthworm activity (i.e. physical and chemical defenses to herbivory and desiccation, deeper rooting, low mycorrhizal dependence). Conversely, many native plants have evolved shallow roots that rely on the unique microhabitat of the O horizon (Huenneke et al. 1990; Corio et al. 2009). Indeed, the presence of earthworms prevents native plant

communities from taking advantage (measured as biomass and species richness) of increased soil fertility in the A horizon (Whitfeld et al. 2014). Considering this facilitation of invasive plants by earthworms in light of previous studies finding earthworms preferentially degrade invasive shrub litter (Heneghan et al. 2007) and removal of invasive shrubs lowers earthworm biomass (Madritch and Lindroth 2009), co-invasion of invasive plants and earthworms may facilitate each other.

In addition to changes in total and extractable soil nutrients, we have demonstrated

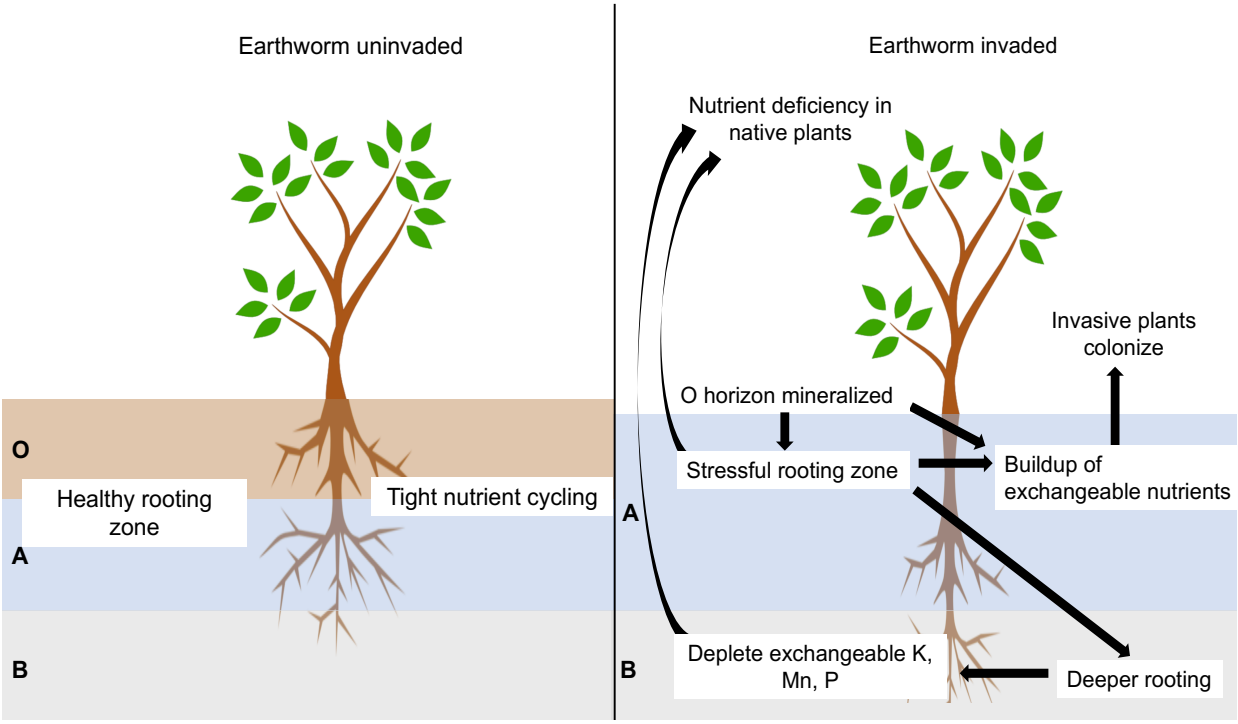


Figure 2-6. Proposed mechanism for earthworm disruption of nutrient cycling in forests.

that earthworm invasion has ramifications for plant nutrition, which may ultimately influence forest understory nutrient dynamics. We hypothesized that earthworm impacts on extractable and total concentrations would influence foliar and root nutrient concentrations. However, our results did not show a consistent, significant relationship between extractable or total nutrient concentrations with root and foliar concentrations. Higher Ca and lower P in both extractable and total soil pools of earthworm-invaded soils were also observed in roots and leaves and among all species (with the exception of Ca in *P. pubescens*) (Figs 3, 4, 6). Similarly, in a P-deficient Quebec forest, *A. saccharum* growing at earthworm-invaded sites had lower foliar P (Paré and Bernier 1989). However, this pattern may be difficult to identify in nutrient-rich forest ecosystems (Suarez et al. 2006b). Higher extractable soil Mg and K concentrations were associated with greater earthworm biomass, but were not associated with plant Mg and K tissue concentration (Figs 3, 4). This disconnect between soil and plant tissue stoichiometry in forest soils is surprisingly common (Bard 1949; Boerner 1986a; Gilliam and Adams 1995; Schaberg et al. 2006). Just as with our foliar tissue, Schaberg et al. (2006) found that while foliar Ca correlated well with soil Ca (Appendix I.4), other nutrients such as K, Mg and Mn did not. Overall, earthworm biomass was a better predictor of foliar nutrients than soil nutrient pools in explaining foliar nutrient concentrations. This could mean that earthworm effects are either more important than soil fertility in plant nutrition, or that earthworm biomass better captures the heterogeneous soil conditions than our soil pits, though these are not mutually exclusive mechanisms.

As ecosystem engineers, it is possible that earthworm invasion overrides site fertility control on plant nutrient dynamics (Fig. 2-6). In addition to indirectly affecting plants by altering soil parameters controlling nutrient availability, earthworm invasion can strain mycorrhizal associations, enhance moisture and temperature stress and directly feed on fine roots (Lawrence et al. 2003; Fisk et al. 2004b; Larson et al. 2010; Gilbert et al. 2014).

Historically, understory plants have maintained remarkably consistent foliar stoichiometry across varying environments through unique evolutionary strategies such as seasonal luxury consumption, root foraging and mycorrhizal associations (Johnson 2010; Hawkesford et al. 2011; Giehl and von Wiren 2014; Gilliam 2014). We propose that the disruption of these strategies by earthworms enhances site-to-site variation in foliar nutrition, which could ultimately explain why earthworms are associated with losses in native species richness, cover and seedling recruitment (Hale et al. 2006b; Holdsworth et al. 2007a; Drouin et al. 2014). Our study provides additional support that earthworms drive physicochemical changes to soil and influence plant nutrition (Fig. 2-6).

Our results show that plant responses to earthworm invasion were variable for different nutrients and species. Many *A. saccharum* seedlings had nutrient concentrations below the lowest value published for healthy foliage, particularly for Ca, Mg, Mn and P (Fig. 2-3; Burton et al. 1993; Kolb, T.E.; McCormick 1993). Nearly all *A. saccharum* seedlings growing in the absence of earthworms are Ca-deficient, reaching healthy Ca concentrations at an earthworm biomass of 4 ± 0.2 g m^{-2}. Many (but not all) are within the range of Mn for healthy foliage, becoming deficient beyond an earthworm biomass of 4 ± 0.2 g m^{-2} (Fig. 2-3). Only one individual (in the earthworm-invaded plots) demonstrated possible Mn toxicity, exceeding healthy Mn levels of 1632 ug kg^{-1} by ~400 ug kg^{-1} (Fig. 2-3; Kolb, T.E.; McCormick 1993). The molar ratio of Ca:Al ranged from 40 to 295 mol mol^{-1}, which is well above the threshold of toxicity where Ca:Al ratios < 9.9 has 50 % chance of Al toxicity (Thornton, F.C., Schaedle, M., Raynal 1986; Cronan and Grigal 1995). Therefore, although many *A. saccharum* seedlings were deficient in several nutrients, Al and Mn toxicity is not a concern.

For both *A. saccharum* and *P. acrostichoides*, earthworm biomass was strongly associated with root and foliar Ca (Figs 3, 4). Tissue Mn concentrations simultaneously

decreased, suggesting that these species may be able to preferentially take up Ca over Mn in earthworm-invaded soils. *Polygonatum pubescens* was the only species that did not have higher tissue concentrations at plots with higher soil Ca and greater earthworm biomass (Figs 3, 4). While root P content was lower with increasing earthworm biomass in all species, *P. pubescens* was also the only species with coinciding declines of P in foliage (Fig 3). Taken together, differences in nutrient availability and uptake may explain this species' susceptibility to earthworm invasion (Dobson and Blossey 2015). Although *P. acrostichoides* thrives with earthworm invasion, earthworm biomass was a negative predictor of foliar Mg, and root K, P and S. It is possible that given their lower nutrient requirements, earthworm impacts on nutrient cycling are less important to fern success compared to angiosperms (Siccama et al. 1970).

Alternatively, Ca could be the most limiting nutrient to *P. acrostichoides*'s growth, such that elevated Ca supersedes the effect of lower concentrations of other nutrients (Bernier et al. 1989; Côté et al. 1995). Foliar nutrients in *A. saccharum* were similar to *P. acrostichoides*, although they are known to be susceptible to earthworms, likely through root herbivory and disruption of mycorrhizae (Lawrence et al. 2003; Fisk et al. 2004b; Corio et al. 2009; Gilbert et al. 2014; Bal et al. 2017). However, the negative impact of earthworms on *A. saccharum* is not universal, and young seedlings may not be affected (Drouin et al. 2014). It is possible that decreases in P in earthworm-invaded plots negate any benefits from higher Ca, or that higher Ca was not enough to elicit a response in this calciphilic species. The large proportion of *A. saccharum* individuals that are potentially deficient in multiple nutrients could support either possibility. Overall, our results suggest native plants differ in their ability to incorporate nutrient resources under earthworm invasion. Species most at risk such as *P. pubescens* are unable to benefit from higher soil Ca in earthworm-invaded forests, while simultaneously becoming more deficient in other macronutrients. This supports previous

research suggesting *P. pubescens*, and species with similar life histories will likely be disproportionately affected by earthworm invasion, and may warrant special conservation concern (Dobson and Blossey 2015).

CONCLUSIONS

In this study, we present evidence that nutrient cycles in forests are altered by earthworm invasion, and earthworms create a different set of selection pressures for plants growing in these forests. Earthworm biomass was a better predictor of plant nutrition than soil nutrient content or concentration. This may be because earthworm invasion overrides site fertility, or that the soil is so heterogeneous that earthworm biomass is a better indicator of site fertility than soil pits. Overall, a plant species' ability to persist or thrive with earthworms may depend on its ability to take advantage of rapidly mineralized nutrients in earthworm-invaded soils.

Our results suggest earthworm invasion creates a stressful rooting zone for many native species, and some understory plants may adapt, while others may disappear. Evidence of the latter has been well documented (Gundale 2002; Hale et al. 2008; Dobson and Blossey 2015), but our observation that rooting volume was highest in the B horizon in the presence of earthworms suggests some plants may simply root deeper (or be replaced by deeper-rooted plants). The lack of shallow roots in earthworm-invaded soils corresponds to a build-up of extractable nutrients. Therefore, early successional and non-native species that can establish successfully in the absence of an organic horizon may benefit from pools of extractable nutrients from mineralization of the organic horizon.

What remains to be seen is consequences for herbivore preference, which can in turn impact plant communities (Hunter et al. 1991; Joern et al. 2012). Impacts on other trophic levels and adjacent ecosystems such as streams and waterways is vitally important to

consider. In addition to macronutrients, earthworm invasions impact micronutrient and metal cycling, which could have ramifications for higher trophic levels (Joern et al. 2012; Richardson et al. 2015; Richardson et al. 2016).

ACKNOWLEDGEMENTS

We thank Tim Fahey, Francoise Vermeylen and Andrea Dávalos for their input in interpreting results and improving data analysis. Juan Pablo Jordan and Wade Simmons helped immensely in field collection, and we are grateful to Gregg McElwee for his help in lab analysis. We would like to thank the New York State Department of Environmental Conservation, Cornell Natural Areas, and Victoria Nuzzo for long-term use of their land for this experiment. This study was conducted through TRP #6673, and we are grateful for funding received through the Mellon Foundation and a Hatch Grant.

WORKS CITED

Asshoff R, Scheu S, Eisenhauer N (2010) Different earthworm ecological groups interactively impact seedling establishment. Eur J Soil Biol 46:330–334. doi: 10.1016/j.ejsobi.2010.06.005

Bal TL, Storer AJ, Jurgensen MF (2017) Evidence of damage from exotic invasive earthworm activity was highly correlated to sugar maple dieback in the Upper Great Lakes region. Biol Invasions. doi: 10.1007/s10530-017-1523-0

Bard GE (1949) The mineral nutrient content of the annual parts of herbaceous species growing on 3 New York soil types varying in limestone content. Ecology 30:384–389.

Berch SM, Kendrick B (1982) Vesicular-arbuscular mycorrhizae of southern Ontario ferns and fern-allies. Mycologia 74:769–776.

Bernier B, Paré D, Brazeau M (1989) Natural stresses, nutrient imbalances and forest decline in southeastern Quebec. Water, Air, Soil Polution 48:239–250.

Bityutskii NP, Lapshina IN, Lukina EI, et al (2002) Role of earthworms in mineralization of organic nitrogen compounds in soil. Eurasian Soil Sci 35:1100–1107.

Boerner REJ (1986a) Seasonal nutrient dynamics, nutrient resorption, and mycorrhizal infection intensity of two perennial forest herbs. Am J Bot 73:1249–1257.

Boerner REJ (1986b) Seasonal nutrient dynamics, nutrient resportion, and mycorrhizal infection intensity of two perennial forest herbs. Am J Bot 73:1249–1257.

Bohlen PJ, Groffman PM, Fahey TJ, et al (2004a) Ecosystem consequences of exotic earthworm invasion of north temperate forests. Ecosystems 7:1–12. doi: 10.1007/s10021-003-0126-z

Bohlen PJ, Pelletier DM, Groffman PM, et al (2004b) Influence of earthworm invasion on redistribution and retention of soil carbon and nitrogen in northern temperate forests. Ecosystems 7:13–27. doi: 10.1007/s10021-003-0127-y

Bohlen PJ, Scheu S, Hale CM, et al (2004c) Non-native invasive earthworms as agents of change in northern temperate forests. Front Ecol Environ 2:427.

Brady NC, Weil RR (2008) Calcium, Magnesium and trace elements. In: The nature and properties of soils, 14th edn. Pearson, Prentice Hall, Upper Saddle River, New Jersey, Columbus, Ohio, pp 639–677

Brundrett MC, Kendrick B (1988) The mycorrhizal status, root anatomy, and phenology of plants in a sugar maple forest. Can J Bot 66:1153–1173. doi: 10.1139/b88-166

Burtelow AE, Bohlen PJ, Groffman PM (1998) Influence of exotic earthworm invasion on soil organic matter, microbial biomass and denitrification potential in forest soils of the northeastern United States. Appl Soil Ecol 9:197–202. doi: http://dx.doi.org/10.1016/S0929-1393(98)00075-4

Burton AJ, Pregitzer KS, Macdonald NW (1993) Foliar nutrients in sugar maple forests along a regional pollution-climate gradient. Soil Sci Soc Am J 57:1619–1628. doi: 10.2136/sssaj1993.03615995005700060036x

Chapman HD (1965) Cation-exchange capacity 1. In: Norman AG (ed) Methods of soil analysis. Part 2. Chemical and microbiological properties. American Society of Agronomy, Soil Science Society of America, Madison, WI, pp 891–901

Chapuis-Lardy L, Le Bayon R-C, Brossard M, et al (2011) Phosphorus in action. Soil Biol 26:295–316. doi: 10.1007/978-3-642-15271-9

Ciesielski H, Sterckeman T, Santerne M, Willery J (1997) A comparison between three methods for the determination of cation exchange capacity and exchangeable cations in soils. agronomie 17:9–16.

Clair SS, Lynch J (2005) Base cation stimulation of mycorrhization and photosynthesis of sugar maple on acid soils are coupled by foliar nutrient dynamics. New Phytol 165:581–590. doi: 10.1111/j.1469-8137.2004.01249.x

Corio K, Wolf A, Draney M, Fewless G (2009) Exotic earthworms of Greak Lakes forests: A search for indicator plant species in maple forests. For Ecol Manage 258:1059–1066. doi: 10.1016/j.foreco.2009.05.013

Côté B, Halloran IO, Hendershot WH, Spankie H (1995) Possible interference of fertilization in the natural recovery of a declining sugar maple stand in southern Quebec. 471–480.

Coulis M, Bernard L, Gérard F, et al (2014) Endogeic earthworms modify soil phosphorus, plant growth and interactions in a legume-cereal intercrop. Plant Soil 379:149–160. doi: 10.1007/s11104-014-2046-4

Cronan CS, Grigal DF (1995) Use of calcium aluminum ratios as indicators of stress in forest ecosystems. J Environ Qual 24:209–226.

Daniel O (1990) Life cycle and population dynamics of the earthworm Lumbricus terrestris L. Swiss Federal Institute of Technology Zurich

De Vleeschauwer D, Lal R (1981) Properties of worm casts under secondary tropical forest regrowth (Nigeria). Soil Sci 132:175–181.

Deleporte S (2001) Changes in the earthworm community of an acidophilous lowland beech forest during a stand rotation. Eur J Soil Biol 37:1–7. doi: 10.1016/S1164-5563(01)01065-2

Dobson A, Blossey B (2015) Earthworm invasion, white-tailed deer and seedling establishment in deciduous forests of north-eastern North America. J Ecol 103:153–164. doi: 10.1111/1365-2745.12350

Dobson A, Blossey B (2014) Data from: Earthworm invasion, white-tailed deer and seedling establishment in deciduous forests of northeastern North America. Dryad Digit. Repos.

Dorning M, Cipollini D (2006) Leaf and root extracts of the invasive shrub, Lonicera maackii, inhibit seed germination of three herbs with no autotoxic effects. Plant Ecol 184:287–296. doi: 10.1007/s11258-005-9073-4

Doust, Jon L. Cavers PB (1982) Sex and gender dynamics in Jack-in-the-Pulpit , Arisaema triphyllum (Araceae). Ecology 63:797–808.

Drouin M, Bradley R, Lapointe L (2016) Forest ecology and management linkage between exotic earthworms , understory vegetation and soil properties in sugar maple forests. For Ecol Manage 364:113–121.

Drouin M, Bradley R, Lapointe L, Whalen J (2014) Non-native anecic earthworms (Lumbricus terrestris L.) reduce seed germination and seedling survival of temperate and boreal trees species. Appl Soil Ecol 75:145–149. doi: 10.1016/j.apsoil.2013.11.006

Edwards CA (2004) Earthworm ecology, 2nd edn. CRC Press, Boca Raton, Florida, USA

Edwards CA, Bohlen PJ (1996) Biology and ecology of earthworms. Chapman & Hall, London, UK

Eisenhauer N, Partsch S, Parkinson D, Scheu S (2007) Invasion of a deciduous forest by earthworms: changes in soil chemistry, microflora, microarthropods and vegetation. Soil Biol Biochem 39:1099–1110. doi: 10.1016/j.soilbio.2006.12.019

Elliott HL (2009) Evaluating the influences of soil calcium and aluminum availability on ecosystem processes in northern hardwood forest. University of Vermont

Ellsworth DS, Liu X (1994) Photosynthesis and canopy nutrition of four sugar maple forests on acid soils in northern Vermont. Can J For Res 24:2119–2127.

Filley TR, McCormick MK, Crow SE, et al (2008) Comparison of the chemical alteration trajectory of Liriodendron tulipifera L. leaf litter among forests with different earthworm abundance. J Geophys Res 113:G01027–G01027. doi: 10.1029/2007JG000542

Fisk MG, Fahey TJ, Groffman PM, Bohlen PJ (2004) Earthworm invasion, fine-root distributions, and soil respiration in North temperate forests. Ecosystems 7:55–62. doi: 10.1007/s10021-003-0130-3

Frelich LE, Hale CM, Scheu S, et al (2006) Earthworm invasion into previously earthworm-

free temperate and boreal forests. Biol Invasions 8:1235–1245. doi: 10.1007/s10530-006-9019-3

Giehl RFH, von Wiren N (2014) Root nutrient foraging. Plant Physiol 166:509–517. doi: 10.1104/pp.114.245225

Gilbert KJ, Fahey TJ, Maerz JC, et al (2014) Exploring carbon flow through the root channel in a temperate forest soil food web. Soil Biol Biochem 76:45–52. doi: http://dx.doi.org/10.1016/j.soilbio.2014.05.005

Gilliam FS (2014) The herbaceous layer in forests of eastern North America, 2nd edn. Oxford University Press, New York, New York, USA

Gilliam FS, Adams MB (1995) Plant and soil nutrients in young versus mature central Appalachian hardwood stands. Proc 10th Cent Hardwood For Conf 109–118.

Gundale MJ (2002) Influence of exotic earthworms on the soil organic horizon and the rare fern Botrychium mormo. Conserv Biol 16:1555–1561. doi: 10.1046/j.1523-1739.2002.01229.x

Hale C, Frelich L, Reich P, Pastor J (2005) Effects of European earthworm invasion on soil characteristics in northern hardwood forests of Minnesota, USA. Ecosystems 8:911–927. doi: 10.1007/s10021-005-0066-x

Hale CM, Frelich LE, Reich PB (2006a) Changes in cold-temperate hardwood forest understory plant communities in response to invasion by European earthworms. Ecology 87:1637–1649. doi: 10.1890/0012-9658(2006)87[1637:CIHFUP]2.0.CO;2

Hale CM, Frelich LE, Reich PB (2006b) Changes in hardwood forest understory plant communities in response to European earthworm invasions. Ecology 87:1637–1649. doi: 10.1890/0012-9658(2006)87[1637:CIHFUP]2.0.CO;2

Hale CM, Frelich LE, Reich PB, Pastor J (2008) Exotic earthworm effects on hardwood forest floor, nutrient availability and native plants: A mesocosm study. Oecologia

155:509–518. doi: 10.1007/s00442-007-0925-6

Hawkesford M, Horst W, Kichey T, et al (2011) Functions of macronutrients. In: Marschner H (ed) Marschner's mineral nutrition of higher plants, 3rd edn. Academic Press, London, UK, pp 135–189

Hendrix P, Bohlen P (2002) Exotic earthworm invasions in North America: ecological and policy implications. Bioscience 52:801–811. doi: 10.1641/0006-3568(2002)052[0801:EEIINA]2.0.CO;2

Heneghan L, Steffen J, Fagen K (2007) Interactions of an introduced shrub and introduced earthworms in an Illinois urban woodland: Impact on leaf litter decomposition. Pedobiologia (Jena) 50:543–551. doi: 10.1016/j.pedobi.2006.10.002

Holdsworth AR, Frelich LE, Reich PB (2007a) Effects of earthworm invasion on plant species richness in northern hardwood forests. Conserv Biol 21:997–1008. doi: 10.1111/j.1523-1739.2007.00740.x

Holdsworth AR, Frelich LE, Reich PB (2007b) Regional extent of an ecosystem engineer: Earthworm invasion in northern hardwood forests. Ecol Appl 17:1666–1677. doi: 10.1890/05-2003.1

Horsley SB, Long RP, Bailey SW, et al (2000) Factors associated with the decline disease of sugar maple on the Allegheny Plateau. Can J For Res 30:1365–1378. doi: 10.1139/x00-057.

Horsley SB, Long RP, Bailey SW, et al (2002) Health of eastern North American sugar maple forests and factors affecting decline. North J Appl For 19:34–44.

Huenneke LF, Hamburg SP, Koide R, et al (1990) Effects of soil resources on plant invasion and community structure in Californian serpentine grassland. Ecology 71:478–491.

Hunter MD, Watt AD, Docherty M (1991) Outbreaks of the winter moth on Sitka Spruce in Scotland are not influenced by nutrient deficiencies of trees, tree budburst, or pupal

predation. Oecologia 86:62–69.

Huntington TG, Hooper RP, Johnson CE, et al (2000) Calcium depletion in a southeastern United States forest ecosystem. Soil Sci Soc Am J 64:1845. doi: 10.2136/sssaj2000.6451845x

James SW (1995) Systematics, biogeography, and ecology of nearctic earthworms from eastern, central, southern and southwestern United States. In: Hendrix PF (ed) Earthworm ecology and biogeography in North America. Lewis, pp 29–52

Jenkins J, Roy K, Driscoll C, Buerkett C (2005) Acid rain and the Adirondacks : a research summary. Ray Brook, New York, USA

Joern A, Provin T, Behmer ST (2012) Not just the usual suspects: Insect herbivore populations and communities are associated with multiple plant nutrients. Ecology 93:1002–1015. doi: 10.1890/11-1142.1

Johnson NC (2010) Resource stoichiometry elucidates the structure and function of arbuscular mycorrhizas across scales. New Phytol 185:631–647. doi: 10.1111/j.1469-8137.2009.03110.x

Juice SM, Fahey TJ, Siccama TG, et al (2006) Response of sugar maple to calcium addition to northern hardwood forest. Ecology 87:1267–1280. doi: 10.1890/0012-9658(2006)87[1267:ROSMTC]2.0.CO;2

Kery M, Gregg KB (2004) Demographic analysis of dormancy and survival in the terrestrial orchid Cypripedium reginae. J Ecol 92:686–695. doi: 10.1111/j.0022-0477.2004.00885.x

Knowles ME, Ross DS, Josef HG (2016) Effect of the endogeic earthworm Aporrectodea tuberculata on aggregation and carbon redistribution in uninvaded forest soil columns. Soil Biol Biochem 100:192–200. doi: 10.1016/j.soilbio.2016.06.016

Kobe RK, Likens GE, Eagar C (2002) Tree seedling growth and mortality responses to

manipulations of calcium and aluminum in a northern hardwood forest. Can J For Res 32:954–966. doi: 10.1139/x02-018

Kolb T, McCormick L (1993) Etiology of sugar maple decline in four Pennsylvania stands. Can J For Res 23:2395–2402.

Kuczak CN, Fernandes ECM, Lehmann J, et al (2006) Inorganic and organic phosphorus pools in earthworm casts (Glossoscolecidae) and a Brazilian rainforest Oxisol. Soil Biol Biochem 38:553–560. doi: 10.1016/j.soilbio.2005.06.007

Laossi K-R, Decaëns T, Jouquet P, Barot S (2010a) Can we predict how earthworm effects on plant growth vary with soil properties? Appl Environ Soil Sci. doi: 10.1155/2010/784342

Laossi KR, Ginot A, Noguera DC, et al (2010b) Earthworm effects on plant growth do not necessarily decrease with soil fertility. Plant Soil 328:109–118. doi: 10.1007/s11104-009-0086-y

Larson ER, Kipfmueller KF, Hale CM, et al (2010) Tree rings detect earthworm invasions and their effects in northern Hardwood forests. Biol Invasions 12:1053–1066. doi: 10.1007/s10530-009-9523-3

Lawrence AP, Bowers MA (2002) A test of the "hot" mustard extraction method of sampling earthworms. Soil Biol Biochem 34:549–552. doi: http://dx.doi.org/10.1016/S0038-0717(01)00211-5

Lawrence B, Fisk MC, Fahey TJ, Suarez ER (2003) Influence of nonnative earthworms on mycorrhizal colonization of sugar maple (Acer saccharum). New Phytol 157:145–153. doi: 10.1046/j.1469-8137.2003.00649.x

Le Bayon RC, Binet F (2006) Earthworms change the distribution and availability of phosphorous in organic substrates. Soil Biol Biochem 38:235–246. doi: 10.1016/j.soilbio.2005.05.013

Leonard JA, Field JB (2004) Distributions of cations in the regolith and vegetation. In: Roach I (ed) Regiolith: Proceedings of the CRC LEME Regional Regolith Symposia 2004. CRC LEME, Bentley, WA, Australia, pp 215–219

Long RP, Horsley SB, Lilja PR (1997) Impact of forest liming on growth and crown vigor of sugar maple and associated hardwoods. Can J For Res 1573:1560–1573.

Madritch MD, Lindroth RL (2009) Removal of invasive shrubs reduces exotic earthworm populations. Biol Invasions 11:663–671. doi: 10.1007/s10530-008-9281-7

McLean M a., Migge-Kleian S, Parkinson D (2006) Earthworm invasions of ecosystems devoid of earthworms: Effects on soil microbes. Biol Invasions 8:1257–1273. doi: 10.1007/978-1-4020-5429-7_7

Newell CL, Peet RK (1998) Vegetation of Linville Gorge Wilderness, North Carolina. Castanea 63:275–322.

Nuzzo VA, Maerz JC, Blossey B (2009) Earthworm invasion as the driving force behind plant invasion and community change in northeastern North American forests. Conserv Biol 23:966–974. doi: 10.1111/j.1523-1739.2009.01168.x

Paré D, Bernier B (1989) Origin of the phosphorus deficiency observed in declining sugar maple stands in the Quebec Appalachians. Can J For Res 19:24–34.

R Core Team (2014) R: A language and environment for statistical computing. 3.0.1:

Reich PB, Oleksyn J, Modrzynski J, et al (2005) Linking litter calcium, earthworms and soil properties: a common garden test with 14 tree species. Ecol Lett 8:811–818. doi: 10.1111/j.1461-0248.2005.00779.x

Resner K, Yoo K, Sebestyen SD, et al (2015) Invasive earthworms deplete key soil inorganic nutrients (Ca, Mg, K, and P) in a northern hardwood forest. Ecosystems 18:89–102. doi: 10.1007/s10021-014-9814-0

Richardson JB, Görres JH, Friedland AJ (2016) Forest floor decomposition, metal

exchangeability, and metal bioaccumulation by exotic earthworms: Amynthas agrestis and Lumbricus rubellus. Environ Sci Pollut Res 23:18253–18266. doi: 10.1007/s11356-016-6994-5

Richardson JB, Gorres JH, Jackson BP, Friedland AJ (2015) Trace metals and metalloids in forest soils and exotic earthworms in northern New England, USA. Soil Biol Biochem 85:190–198. doi: 10.1016/j.soilbio.2015.03.001

Royo AA, Carson WP (2008) Direct and indirect effects of a dense understory on tree seedling recruitment in temperate forests: habitat-mediated predation versus competition. Can J For Res 38:1634–1645. doi: 10.1139/X07-247

Schaberg PG, Tilley JW, Hawley GJ, et al (2006) Associations of calcium and aluminum with the growth and health of sugar maple trees in Vermont. For Ecol Manage 223:159–169. doi: 10.1016/j.foreco.2005.10.067

Schelfhout S, Mertens J, Verheyen K, et al (2017) Tree species identity shapes earthworm communities. Forests 8:85. doi: 10.3390/f8030085

Scheu S (1994) There is an earthworm mobilizable nitrogen pool in soil. Pedobiologia (Jena) 38:243–249.

Scheu S, Parkinson D (1994) Effects of earthworms on nutrient dynamics, carbon turnover and microorganisms in soils from cool temperate forests of the Canadian Rocky Mountains laboratory studies. Appl Soil Ecol 1:113–125.

Siccama T, Bormann F, Likens G (1970) The Hubbard Brook Ecosystem study: productivity, nutrients, and phytosociology of the herbaceous layer. Ecol Monogr 40:389–402.

St. Clair S, Lynch JP (2005) Element accumulation patterns of deciduous and evergreen tree seedlings on acid soils: Implications for sensitivity to manganese toxicity. Tree Physiol 25:85–92. doi: 10.1093/treephys/25.1.85

Suarez ER, Fahey TJ, Yavitt JB, et al (2006a) Patterns of litter disappearance in a northern

hardwood forest invaded by exotic earthworms. Ecol Appl 16:154–165. doi: 10.1890/04-0788

Suarez ER, Pelletier DM, Fahey TJ, et al (2004) Effects of exotic earthworms on soil phosphorus cycling in two broadleaf temperate forests. Ecosystems 7:28–44. doi: 10.1007/s10021-003-0128-x

Suárez ER, Pelletier DM, Fahey TJ, et al (2004) Effects of exotic earthworms on soil phosphorus cycling in two broadleaf temperate forests. Ecosystems 7:28–44. doi: 10.1007/s10021-003-0128-x

Suarez ER, Tierney GL, Fahey TJ, Fahey R (2006b) Exploring patterns of exotic earthworm distribution in a temperate hardwood forest in south-central New York, USA. Landsc Ecol 21:297–306. doi: 10.1007/s10980-005-1785-2

Szlavecz K, McCormick M, Xia L, et al (2011) Ecosystem effects of non-native earthworms in Mid-Atlantic deciduous forests. Biol Invasions 13:1165–1182. doi: 10.1007/s10530-011-9959-0

Szlavecz K, Placella SA, Pouyat R V, et al (2006) Invasive earthworm species and nitrogen cycling in remnant forest patches. Appl Soil Ecol 32:54–62. doi: 10.1016/j.apsoil.2005.01.006

Thornton, F.C., Schaedle, M., Raynal DJ (1986) Effect of aluminum on growth of sugar maple in solution culture. Can J For Res 16:892–896.

Umarov MM, Striganova BR, Kostin N V (2008) Specific features of nitrogen transformation in the gut and coprolites of earthworms. Biol Bull 35:643–652. doi: 10.1134/S1062359008060125

Vos HMJ, Ros MBH, Koopmans GF, van Groenigen JW (2014) Do earthworms affect phosphorus availability to grass? A pot experiment. Soil Biol Biochem 79:34–42. doi: 10.1016/j.soilbio.2014.08.018

Weihua D, Xiuqin Y (2007) Transformation of carbon and nitrogen by earthworms in the decomposition processes of broad-reaved litters. Chinese Geogr Sci 17:166–172. doi: 10.1007/s11769-007-0166-y

West B, Brandt J, Holstien K, et al (2009) Fern-associated arbuscular mycorrhizal fungi are represented by multiple Glomus spp.: Do environmental factors influence partner identity? Mycorrhiza 19:295–304. doi: 10.1007/s00572-009-0234-5

Whitfeld TS, Roth A, Lodge A, et al (2014) Resident plant diversity and introduced earthworms have contrasting effects on the success of invasive plants. Biol Invasions 16:2181–2193. doi: 10.1007/s10530-014-0657-6

Wironen M, Moore TR (2006) Exotic earthworm invasion increases soil carbon and nitrogen in an old-growth forest in southern Quebec. Can J For Res Can Rech For 36:845–854. doi: 10.1139/X06-016

Wood T, Bormann FH, Voigt GK (1984) Phosphorus cycling in a northern hardwood forest: biological and chemical control. Science (80-) 223:391–393.

Yanai RD (1992) Phosphorus budget of a 70-year-old northern hardwood forest. Biogeochemistry 17:1–22.

Zhang BG, Li GT, Shen TS, et al (2000) Changes in microbial biomass C, N, and P and enzyme activities in soil incubated with the earthworms Metaphire guillelmi or Eisenia fetida. Soil Biol Biochem 32:2055–2062. doi: 10.1016/S0038-0717(00)00111-5

Chapter 3

DIRECT AND INDIRECT EFFECTS OF EARTHWORMS AND WHITE-TAILED DEER ON FOREST SOILS, ROOT ARCHITECTURE, SEEDLING PERFORMANCE AND ARBUSCULAR MYCORRHIZAL COLONIZATION

ABSTRACT

Northeastern North American forests experience multiple co-occurring stressors including earthworm invasions and high abundance of white-tailed deer (*Odocoileus virginianus*). Understory plant composition changes and diversity declines under these altered conditions, but decisive mechanisms of impact beyond direct browse by deer remain elusive. Here, we used experimental transplants of five native understory plant species (*Actaea pachypoda, Aquilegia canadensis, Cornus racemosa*, *Quercus rubra* and *Prenanthes alba)* in an existing network of a 2 x 2 factorial experiment at five forested sites to test several mechanistic hypotheses. We expected deer and earthworms to directly inhibit seedling growth and survival. In addition, we tested indirect pathways for this reduction, including decreased soil nutrient concentrations and pools of N and P, root architecture (lower proportion of total root length in roots <0.25mm diameter) and less abundant and active root colonization by arbuscular mycorrhizal fungi (AMF) . We developed structural equation models (SEM) to identify the specific pathways through which earthworms and deer were impacting plant species with different life histories. The presence of earthworms was associated with lower biomass and survival of *Actaea* and survival in *Quercus* and *Prenanthes*, but increased survival of *Cornus*. Deer reduced *Actaea* and *Cornus* survival, but were associated with higher survival of *Prenanthes*. Deer decreased total N concentrations and both deer and earthworms decreased total soil P concentrations. Pools of extractable P and total N in the top 20cm of soil were smaller in earthworm-invaded plots, but were unaffected by deer. Earthworms decreased the proportion of fine roots in *Actaea* and *Cornus*, but increased fine roots in *Aquilegia*. Percent colonization by AMF was only decreased by earthworms in

Quercus transplants, but was otherwise only strongly affected by total N concentration/pools and extractable P pools. Our SEMs showed high correlation among soil variables, but because we do not know which variables are the drivers of this change and which are the passengers, we can only conclude that they are changing together with deer and earthworm influence. Despite earthworm and deer effects on soil fertility, root architecture and limited effects on AMF colonization, plant species showed variable responses in survival and biomass through these multi-step paths.

INTRODUCTION

North American forests are experiencing change from multiple stressors such as land use change, pollution, loss of predators, species invasions and climate change (Wiegmann and Waller 2006; Dávalos et al. 2014). Identifying the mechanisms that drive this change is challenging because these forces are usually co-occurring (Fisichelli et al. 2013; Dávalos et al. 2015a; Simmons et al. 2015). To preserve biodiversity and ecosystem function, we must disentangle individual and combined effects of these co-occurring stressors to implement the best conservation and management strategies (Didham et al. 2005; Grarock et al. 2014; Côté et al. 2016). Consequences of co-occurring stressors can be particularly challenging to identify when they involve both above and belowground processes (Jing et al. 2015; Kuebbing et al. 2015).

Herbivore population expansions and the spread of invasive species are part of a global change framework that affects primary producers (Rooney and Waller 2003; Hendrix et al. 2008; Seki et al. 2014). In North American forests, white-tailed deer (*Odocoileus virginianus* Zimmermann) and invasive earthworms are two stressors thought to severely limit native understory plant populations (Bohlen et al. 2004a; Frerker et al. 2014). Since the Wisconsin glaciation, earthworms have not been present in northeastern North American forests, but have since arrived from Europe and Asia, spreading to remote areas of the continent through human activity (James 1995; Burtelow et al. 1998). White tailed deer, while native to North America, have grown to historically unprecedented densities (Fletcher et al. 2001; Côté et al. 2004). Notably, it is often plant species with similar traits (palatable, highly mycorrhizal, perennial, slow growing forest obligates) that are most affected by both deer and earthworms (Gundale 2002; Hale et al. 2006a; Frelich et al. 2006; Dobson and Blossey 2015). Although the most impacted species have many shared traits, a clear mechanism has not emerged to link above and belowground processes. Furthermore,

sustained high deer populations can facilitate earthworms, potentially confounding studies that do not account for independent effects of these stressors (Dávalos et al. 2015b). In the present study, we aim to test the direct and indirect (soil nutrients, mycorrhizal mutualisms, root architecture) effects of deer and earthworms on understory seedlings and understand how the indirect pathways interact with each other.

Deer browse palatable species, often consuming the largest individuals that contribute most to the next generation (Waller 2014; Nuzzo et al. 2017). However, deer have additional indirect effects beyond browsing (Heckel et al. 2010; Shelton et al. 2014; Dobson and Blossey 2015). Recently, the influence of high deer population on below-ground community composition and soil characteristics have been investigated as mechanisms of indirect effects on native plant communities (Vitousek et al. 2002; Heckel et al. 2010; Kluber et al. 2012; Shelton et al. 2014; Dávalos et al. 2015b). For vertebrate herbivores generally, we expect increased soil fertility by browsing and redistributing nutrients (Doughty 2017; Eisenhauer 2017; Sobral et al. 2017). Furthermore, dominant plant species may respond to herbivory with compensatory growth, leading to greater nutrient inputs (Augustine and McNaughton 1998). However, at high densities, deer can remove considerable green foliage, and may use leaf litter as an alternative food source in winter months when green forage is not available (Johnson et al. 1995). Decomposition can be further accelerated by deer mechanically breaking up litter, increasing leaf contact with soil detritivores and facilitating decomposition (Heckel et al. 2010; Bressette et al. 2012). Ungulates such as deer can increase soil compaction, either through trampling or disruption of fine roots and fungal hyphae (Rossow et al. 1997; Heckel et al. 2010; Shelton et al. 2014). Therefore, we expect deer to increase nutrient loss from our soils, inhibit fine root growth and mycorrhizal colonization.

Earthworm activity can have impacts that mirror that of high deer density, such as accelerated leaf litter decomposition (Peterson et al. 2001; Nuzzo et al. 2009; Fahey et al.

2013). In cold, acidic forest soils such as ours, this occurs through physical mixing of litter and priming decomposition of recalcitrant soil organic matter (SOM) (Fahey et al. 2013). Earthworms alter biotic communities involved in decomposition by limiting arthropod communities and facilitating bacteria over fungi (Bonkowski et al. 2000; McLean and Parkinson 2000; McLean et al. 2006; Eisenhauer 2010; Dempsey et al. 2011). Accelerated decomposition may ultimately lead to the complete loss of the organic horizon, causing stressful rooting conditions such as less insulation from temperature extremes (Larson et al. 2010). The loss of the organic horizon affects soil water dynamics, increasing evaporation rates, amplitude and frequency of drought in times of low precipitation and erosion and runoff in times of high precipitation (Francis and Fraser 1998; Larson et al. 2010). Although earthworm impacts on soil nutrient concentrations depend on soil type, nutrient identity, sampling depth and earthworm community composition (Suarez et al. 2004; Bohlen et al. 2004b; Hale et al. 2005; Marhan and Scheu 2006; Resner et al. 2015), we expected earthworms to be associated with greater losses of soil nutrients in our plots through the mineralization and mixing of the organic horizon.

Whereas deer preferentially browse larger understory plant individuals, earthworms may consume roots and young seedlings (Fisk et al. 2004a; Kirchberger et al. 2015). Estimates from forests in the same region as our study sites suggest earthworms consume ~14% of fine root biomass annually (Gilbert et al. 2014). Although earthworms can act as dispersal vectors for spores, they consume fungal hyphae and physically disrupt networks, leading to decreases in fungal species diversity, density and richness (Rabatin and Stinner 1988; Lawrence et al. 2003; Bohlen et al. 2004a; McLean et al. 2006; Paudel et al. 2016). Impacts on fungal communities could be especially problematic in northeastern North American forests where in response to deep organic horizons, many plant species are highly dependent on mycorrhizal associations and have evolved thick roots with a large cortex in

support of greater rates of colonization (Wang et al. 2017). The combination of stressful growing conditions and direct feeding by earthworms may disproportionately affect fine roots and arbuscular mycorrhizal fungi (AMF), which are vitally important for plant nutrient uptake and water relations (Baylis 1970; Bardgett and Putten 2014; McCormack et al. 2015). Stress on this mutualism may manifest as either lower AMF colonization, fewer arbuscules or more vesicles (potentially caused by carbon stress) (Lawrence et al. 2003; Paudel et al. 2016b). With earthworms creating a more stressful rooting environment and consuming hyphae and fine roots, we expect seedlings in earthworm-invaded plots to have diminished mycorrhizal colonization and fewer fine roots.

In addition to direct impacts by deer and earthworms, soil nutrients, AMF and fine roots influence each other. We expect soil P and AMF to be inversely correlated, as plant P status regulates AMF symbiosis, and AMF may in turn affect soil P by solubilizing organic soil P (Bolan 1991; Smith et al. 2011). The effect of N in AMF colonization is more complicated, and it may be a signaling molecule for AMF mutualisms (Cappellazzo et al. 2007; Bonneau et al. 2013). There is evidence that both plant N deficiency or organic N surplus promote mycorrhiza formation in different experiments, and co-limitation of N and P may increase AM colonization (Blanke et al. 1991; Johansen et al. 1994; Olsson et al. 2002; Hodge and Fitter 2010; Bonneau et al. 2013). Root architecture and soil P are intrinsically related because P uptake occurs primarily through diffusion, making roots growing in P-depleted soils energetically costly and disadvantageous (Kramer-Walter et al. 2016). One strategy employed by some plant species is to modify root architecture and grow long, thin roots to identify P-rich microhabitats (Laliberte et al. 2015). Conversely, a thicker root cortex with larger cortical cells might support higher metabolic rates (lower respiration, greater CO_2 assimilation, faster growth) and greater pathogen resistance in nutrient-rich soils (Chimungu et al. 2014; Laliberte et al. 2015). Both fine roots and mycorrhizae are important for plant

nutrient and water relations, but we expect to see a negative correlation between these two variables due to the evolutionary tradeoff between development of root hairs and dependence on AMF mutualisms (Baylis 1970; Brundrett 2009). If this tradeoff occurs as a result of earthworm disruption to AMF mutualisms, it could suggest a plant has the plasticity to grow fine roots to compensate for lower AMF colonization where deer and earthworms create poor conditions for mycorrhiza (Baylis 1970).

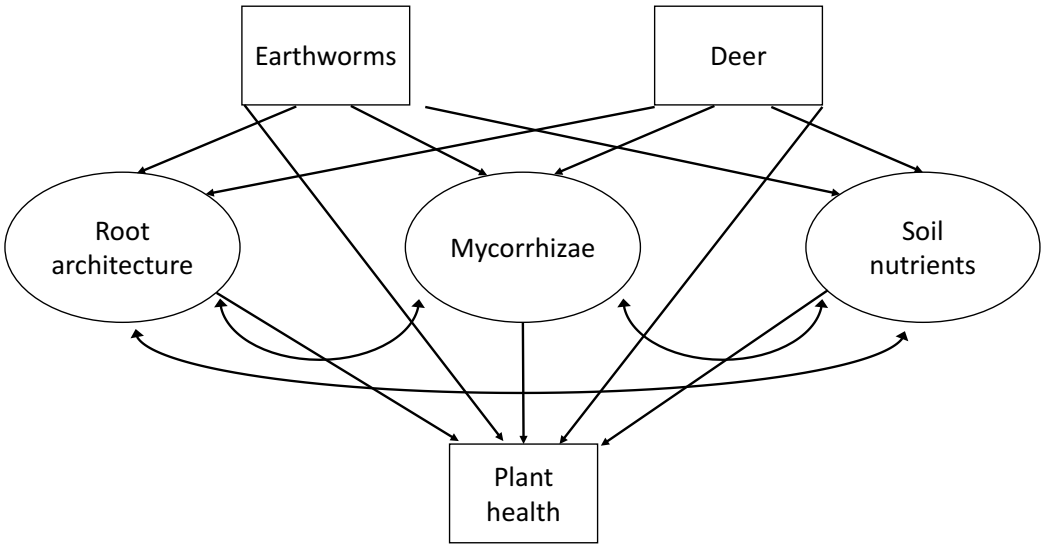


Figure 3-1. Conceptual structural equation models of direct (earthworms and deer) and indirect [root architecture (proportion <0.25mm diameter), AM colonization and soil nutrient] predictors of seedling survival and biomass. Single-headed arrows reflect causative paths and double headed arrows reflect covariance (correlated errors).

To test how these stressors act in isolation and in combination we designed this study within an existing 2 x 2 factorial experiment with paired fenced and unfenced plots within paired earthworm invaded/uninvaded locations (Dobson and Blossey 2015; Dobson et al. 2017a).We used transplants of five native plant species to standardize the age and species pools across several sites. We modeled seedling performance using structural equation models (SEMs), which are well suited to test multiple, non-mutually exclusive hypotheses for the mechanisms of impact. SEMs can capture compensatory shifts, such as plants relying on

fine roots where conditions for AM colonization are poor (Baylis 1970). Here, we use SEM to understand the relative contributions of direct and indirect (changes to soil, mycorrhizae and root architecture) effects of deer and earthworms on survival of native understory seedlings (Fig. 2-1). We aim to test whether (1) Earthworms and deer directly reduce seedling survival and growth; (2) Earthworms and deer reduce seedling survival and growth by decreasing soil nutrient concentrations and pools (3) Earthworms and deer reduce seedling survival and growth by limiting growth or persistence of fine roots (4) Earthworms and deer reduce seedling survival and growth by disrupting plant-AMF mutualisms causing reduced colonization and a higher proportion of vesicles relative to arbuscules.

METHODS AND MATERIALS

Study area

This experiment was conducted at a network of sites (Bobolink Hill, Connecticut Hill Wildlife Management Area, Hammond Hill State Forest, Ringwood Preserve and Yellow Barn State Forest) as part of a long-term experiment to test multiple stressors on forest understory communities (Dobson and Blossey 2015). Forests are in the Allegheny section of the Appalachian Plateau at approximately 42°N, 76°W, with acidic (pH 3.9 – 5.0), soils are Fragiaquepts and Dystrochrepts in the Mardin and Volusia series derived from glacial till, Devonian shale and siltstone (SoilWeb, USDA-NRCS & UC Davis California 2010). Canopies at all forested sites are dominated by mature *A. saccharum, Fraxinus* spp., *Fagus grandifolia* Ehrh. and *Quercus rubra* L. (Leaf Area Index = 5-7). Forests ranged from some timber harvest (Ringwood) to actively (Connecticut Hill, Yellow Barn) and passively (Hammond Hill, Bobolink Hill) reforested farm and grazing land. Deer were nearly eliminated in the early 20th century, but have recolonized and flourished since the mid 1900's (Halls 1984). While we do not know deer abundance or population fluctuations in our forests, deer densities in the Finger Lakes region typically range from 3.6 – 11.6 deer km^{-2} , but can

reach 22 deer km^{-2} (Hunn 2007; Boulanger et al. 2014; Russell et al. 2017). Similarly, earthworm-invasion histories are unknown, however all plots at Ringwood, Yellow Barn and Bobolink Hill retained traces (<5 cm depth) of an organic horizon. Detailed soil, land use history and environmental variables about the study site are discussed in Dobson and Blossey (2015), Dobson et al. (2017), and Richardson et al. (2018).

In 2011, we created a 2 x 2 factorial design replicated at each forest to assess effects of white-tailed deer and invasive earthworms on native understory plant communities. We selected two locations in each forest, one with an existing earthworm invasion and a second in an earthworm-free area (0.5–2 km between locations). At each location, we created a 50 x 50 m deer exclusion plot using a 2.5-m-high plastic mesh fence (deerbusters.com, Standard perimeter fencing, Waynesboro, PA) held upright by parallel cables secured to trees. Adjacent to the fenced plot, we delineated a control plot where deer had access (for further details see Dobson and Blossey 2015).

Seedling establishment

We selected five species to represent a breadth of life histories (growth form, mycorrhizal dependence, root architecture) and hypothesized responses to deer and earthworms. We collected seed from local (within 100 km) sources, and germinated seedlings of *Actaea pachypoda* Elliott and *Cornus racemosa* Lam. in summer 2013, and *Aquilegia canadensis* L., *Quercus rubra* L., *Prenanthes alba* L. in early spring 2014. Those seedlings germinated in 2013 were held in a cold frame over the winter. All species support AMF mutualisms, although *Quercus* associates primarily with ectomycorrhizal (EM) fungi (Dickie et al. 2001; Toju et al. 2014). We germinated seedlings in potting soil (BX General Purpose Pro-mix, Premier Brands Inc., Riviere-du-Loup, Quebec, Canada). We selected the most vigorous individuals to transplant into new potting soil in 6 x 3.7 x 6 cm cell packs. *Quercus* were

germinated from acorns in the spring directly into 3.8 cm diameter Conetainers (Stuewe and Sons, Corvallis, Oregon, USA). We grew seedlings outdoors under 1.8 x 1.8 x 3.6 m shade tent (Lumite®; Alto, Georgia, USA) to protect from deer and elevated in 2 mm nylon mesh reptarium cages (Reptarium™ 65 gallon [41 x 75 x 70 cm], Dallas MFG Co. Dallas, Texas) on a steel mesh table with legs submerged in soapy water to prevent earthworm access.

In May 2014, we rinsed potting soil from roots and planted bare-root seedlings into assigned, randomized planting locations in the forested plots. As we rinsed them, we did not observe any earthworms in potting soil, suggesting the setup restricted earthworm access. Circular plot with a 7m diameter were randomly selected from within existing 50 m^2 plots that are part of a larger experiment testing earthworm and deer impacts (Dobson and Blossey 2015). Within each plot, individually marked planting locations were set up 1m apart around the perimeter of the plot. Each plot contained three to five individuals of each species. In late August/early September 2014 we carefully extracted seedlings including their root ball using a planting knife (Professional Gardener's Digging Tool, Garret Wade, Cincinnati, OH), to retain fine roots. We considered an individual surviving if it was visibly alive above leaf litter. While it is possible some individuals were dormant, we will henceforth refer to the visibly living individuals as Surviving. We separated roots from shoots in the field, drying the latter in a greenhouse for two weeks and then determined dry biomass. Due to our destructive sampling of roots for other analyses, we used only the aboveground portion of the dry biomass in our analyses. Henceforth, we will use biomass to describe dry aboveground biomass. We removed all soil from roots through soaking and cleaning with a paintbrush, weighed them (wet) and immediately transferred roots to 70% ethanol.

Soil Sampling

We measured nutrient concentrations (nutrient mass per soil mass) and pools (nutrient mass per volume fine fraction soil) to a depth of 20 cm. We obtained soil subsamples from soil monoliths as detailed by Dobson and others (2017). In each plot, one 15 cm^2 soil monolith was dug in summer 2016 at a random location in each plot at four forested sites (excluding Connecticut Hill). Monoliths included organic horizon, where present, but excluded Oi litter. Because no samples were excavated from Connecticut Hill, we did not analyze nutrient effects on seedlings from that site. We separated, air dried and sieved the A-horizon to < 2 mm. We obtained %N soil concentration from an elemental analyzer through Cornell University's Stable Isotope Laboratory (COIL). For extractable P concentrations, we suspended 2 g of soil in 20 mL of 1 M ammonium acetate by shaking samples for 1 hr and allowing samples to equilibrate for 24 hrs (Chapman 1965; Ciesielski et al. 1997). We centrifuged soil slurries at 3000 rpm for 30 min and decanted the extraction. We performed a pseudo-total (henceforth referred to as total) digestion to measure total P concentrations following EPA method 3051A. Total digests did not include dissolve silicates and other refractory compounds. We digested 0.5 grams of air-dried material in 5 ml of 8 M reverse aqua regia (9:1, HNO_3 : HCl) at 90°C for 45 min on an insulated hot plate in sealed teflon vials. We analyzed diluted digestate via ICP-OES (SPECTRO Analytical Instruments, Kleve, Germany). We included a digestion blank for every 25 samples as well as matching standard reference materials (SRM) for extractable and total P concentrations (Montana Soil 2711 from the National Institute of Standards and Technology, Gaithersburg, MD). R elative standard deviations (RSDs) of SRMs were less than < 14 % for total soil concentrations and < 18 % for extractable soil concentrations. Blanks had elemental concentrations below detection limit for P (50 ug L^{-1}). Total concentration recoveries for Montana Soil SRM were 62 - 96 % for P, likely due to the silicate minerals and other residual compounds insoluble in concentrated HNO3 and HCl.

Root architecture

To assess the proportion of total root length that was fine roots <0.25mm in diameter, we captured 2D images of whole seedling roots in 70% isopropyl alcohol using a photo scanner (Epson Expression 10000XL, 240 dpi, Epson America Inc., Long Beach, CA). Where possible we manually separated individual roots to prevent overlapping segments. We analyzed root length by diameter, measuring the length of all roots above and below 0.25mm diameter (Regent Instruments, Quebec, Canada). We chose the <0.25mm diameter class to most accurately capture the finest, highest order roots of our one-year old seedlings. Our root architecture variable was the total length of roots <0.25mm in diameter divided by the total root length of the sample. Although root diameter is a poor predictor of root lifespan or plant growth rate (Smith et al. 2014; Kramer-Walter et al. 2016), it is a reliable general predictor of other root traits associated with soil fertility such as Specific Root Length (SRL) and % root nitrogen (Kramer-Walter et al. 2016; Wang et al. 2017).

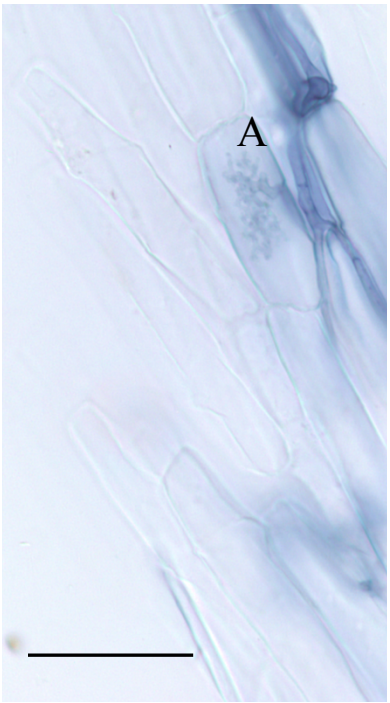


Figure 3-2. Arbuscular mycorrhizal associations including arbuscules (A) in *Cornus*. Bar = 50 µm.

Mycorrhizal colonization

We assessed mycorrhizal colonization through staining. We transferred whole roots from smaller seedlings or the lower 5 cm of larger seedlings into 50 mL Falcon tubes with 10 % KOH (w/v). Roots varied among species ranging from delicate to woody and pigmented, hence we treated each species differently, heating samples to 80◦C for 2 hours – 7 days and replacing the KOH solution between one and six times. After clearing, we rinsed samples three times in 5%

HCl. For heavily pigmented species (*Actaea, Cornus, Quercus*), we treated samples with 0.5% NH4OH and 0.5% H_2O_2 for 24 hours, followed by a triple wash in 5% HCl. We subsampled roots to confirm sufficient clearing under a dissecting scope (MZ6, Leica Microsystems, Buffalo Grove, IL). We stained samples in 5% blue ink (Parker Quink Ink, Atlanta, Georgia) diluted in 5% acetic acid (Vierheilig et al. 1998) for 48 hours. We transferred roots to a 50% glycerol 50% deionized water solution for de-staining for an additional 48 hours. Next, we cut ~ 20 root tips from each individual and trimmed to ~ 1 cm lengths. We squash mounted root segments in a row along a microscope slide to quantify endophytic fungi. Using the magnified intersection method (McGonigle et al. 1990), we categorized 50 (*Prenanthes* and *Quercus*, which had limited root tissue) and 100 (all other species) points using a microscope (40X – 1000X Infinity Plan EPI, Leica Microsystems, Buffalo Grove, IL) along root segments as: 1) AM hyphae present 2) dark septate endophytes (DSE) present 3) unknown hyphae present and 4) no fungal colonization (Fig. 2-2). Within samples with AM hyphae, we quantified presence of arbuscules, vesicles and hyphal coils (Fig. 3-2) (Brundrett et al. 1996). We used the % root length with hyphal coils, arbuscules or both to represent mycorrhiza (and refer to them henceforth as AM colonization) because they are the regions of active mutualism (McGonigle et al. 1990; Brundrett 2004; Dickson 2004). We identified DSE by the presence of melanized microsleclerotia (aggregate irregularly lobed hyphae) that do not stain and dark septate hyphae in the host root (Stoyke and Currah 1991; Brundrett et al. 1996; Mandyam and Jumpponen 2005; Luke et al. 2015).

Statistical analysis

To understand direct and indirect mechanisms for deer and earthworm effects on seedling survival and biomass of surviving individuals we used structural equation models (SEMs). SEMs are multivariate probabilistic path analyses that can test a suite of interrelated variables

in one unified network (Grace 2006). They are particularly useful to disentangle causation from correlations that arise as a result of common responses to an experimental manipulation (Chaudhary et al. 2009; Eisenhauer et al. 2015). Traditional SEM assumes independence of observations, and does not allow for random variables or nested structures (Grace 2006; Lefcheck 2015). By building models piecewise, we are able to include site as a random effect to prevent pseudoreplication using piecewiseSEM and lavaan (Lefcheck 2015; R Core Team 2016). piecewiseSEM is graph theoretic approach by which a path diagram is translated to a set of structured linear equations. Unlike traditional SEM, each component model is evaluated separately, allowing for the fitting of diverse models (Shipley 2009; Lefcheck 2015).

For each component model of each species' SEM, we included the initial height of seedlings at time of transplanting and fit the random effect of site (allowing the intercept only to vary). These models included deer and earthworms (presence/absence) as exogenous variables on all endogenous variables. Endogenous variables are influenced by other variables (including other endogenous variables), while exogenous variables are not influenced by any other variables in the model (Grace 2006; Eisenhauer et al. 2015). For simplicity, implicit in the model is the assumption that deer and earthworms' influence on endogenous variables is unidirectional. Furthermore, although there is evidence than deer exclusion decreases earthworm abundance (Rearick et al. 2011; Shelton et al. 2014; Dávalos et al. 2015b), because deer and earthworms were categorical variables, we run our models with the assumption that deer and earthworms are not influencing each other.

We included all indirect pathways including deer and earthworm effects on total N, extractable P, total P, AMF colonization (length/length), initial height/width (depending on species), fine root:total root length), and the effect of these parameters on seedling biomass. We ran separate SEMs for each plant species, both measure of soil nutrients (concentration

and pools), as well as seedling performance (biomass and survival). We assessed covariance (correlated errors) in the pathways that we did not manipulate experimentally or have explicit hypotheses for. Because colonization of DSE was low, we analyzed presence/absence instead. We then looked for missing paths using Shipley's test of directed separation (*d-sep*)(Shipley 2000). By testing the assumption that there are no missing relationships among variables, we confirmed all variables are conditionally independent, which we represent with Fisher's C statistic (Shipley 2000). We use Fisher's C to obtain model-network P-values and calculate AIC and AICc. P-values are related to the null hypothesis that the model does not reproduce the data, in other words $p > 0.05$ represents a good model (Lefcheck 2015). We tested for significant paths using unstandardized data, but present regression coefficients standardized by mean and variance for SEMs for comparison (Lefcheck 2015). We calculated all regression coefficientss and marginal and conditional R^2 (individual model fits) from residual maximum likelihood (REML).

In addition to SEMs, we used linear mixed models to test whether earthworms and deer had an impact on the absolute and relative number of vesicles in root samples. We analyzed each plant species separately, with site as a random effect and an earthworm biomass x fence interaction a fixed effect.

Table 3-1. Range, mean or median of variables, number of transplants (n) and traits of each plant species at each site (n=5 sites). We present means for parametric data and median for non-parametric data.

		Actaea	*Aquilegia*	*Cornus*	*Prenanthes*	*Quercus*
Growth form		Herb	Herb	Shrub	Herb	Tree
Root		Rhizome	Fibrous	Fibrous, Suckering	Taproot	Woody taproot
Growth rate		Slow	Fast	Slow	Fast	Slow
N planted$^{\alpha}$	n	80	60	80	80	100
Survival$^{\beta}$	Range (%)	50 - 100	0 - 100	25 - 100	0 - 100	0-100
	Mean (%)	86	63	89	45	58
Dry Biomass$^{\chi}$	Range	0.01 - 2.19	0.01 - 0.76	0.03 - 0.31	0.01 - 0.2	0.2-1.6
	Mean	0.37	0.16	0.1	0.04	0.68
Mycorrhizal colonization (%)$^{\delta}$	Range	6 to 96	0 - 84	0 - 68	0 - 96	4 to 88
	Mean	38	44	28	40	37
Length < 0.25mm Total root length$^{\varepsilon}$	Range	0.19 - 0.55	0.54 - 0.98	0.31 - 0.76	0.19 - 0.79	0.36 - 0.67
	Mean	0.37	0.65	0.51	0.53	0.52
DSE$^{\phi}$	Range	0 - 10	0 - 48	0 - 42	0 - 8	0 - 56
	Median	0	4	0	0	16

α Total transplants in the experiment.

β Any seedlings present above the leaf litter at the time of the experiment.

χ Dry biomass of aboveground plant tissue.

δ % colonization (length/length) by arbuscular fungi. Confirmed by the presence of arbuscules and/or hyphal coils.

ε Proportion of root length considered fine roots (diameter < 0.25 mm) relative to total root length.

ϕ % colonization (length/length) by dark septate endophytes. Confirmed by the presence of melanized microsleclerotia.

RESULTS

Seedling performance

Seedling survival varied among both plots and species, and our models explained 69 – 97% of the variance (Table 3-1; Figs 3-3a,c – 3-7a,c; APPENDIX II.9 & II.25). Earthworms reduced *Actaea*, *Quercus* and *Prenanthes* survival, but had a positive effect on *Cornus* (Figs 3-5a,c – 3-7a,c; APPENDIX II.12 – 14 & II.28 - 30). Deer reduced *Actaea* and *Cornus* survival, but increased the survival of *Prenanthes* (Fig 3-5; APPENDIX II.12, II.28). Extractable P (but not total soil P) concentrations was associated with lower survival in all species, but higher survival in *Quercus* (Fig 3-7; APPENDIX II.14 & II.30). *Aquilegia, Quercus* and *Prenanthes* had lower survival with increasing total P concentration. Species had variable responses to total N concentration, ranging from increased (*Quercus*) to decreased (*Cornus*) survival. AM colonization enhanced *Quercus* survival, while the proportion of fine roots had no effect. In contrast, total P pools (in the top 20 cm of soil) were associated with lower survival of *Actaea* and *Prenanthes*, while exchangeable P pools were associated with higher survival in *Cornus, Quercus* and *Prenanthes*. Total N pools were associated with lower survival in *Actaea* and *Quercus*.

Our models explained 5 - 53% of the variance in dry aboveground biomass (Table 3-1; Figs 3-3b,d – 3-7b,d; APPENDIX II.1&II.17). However, the biomass individual seedlings produced over a single growing season was extremely variable, spanning orders of magnitude even within a species (Table 3-1). Surprisingly, initial height at planting was not associated with survival or biomass at the end of the experiment, therefore we removed it from our models. *Actaea* biomass decreased with higher earthworm biomass and total N, but increased in the presence of larger pools of extractable P (Figs 3-3b,d; APPENDIX II.1&II.17).

Soil nutrient concentrations and pools

Using our concentration models, it was possible to explain 20 - 25% of variance in extractable P, 60 - 65% in total P and 65 - 70 % in total N. Soil pool models explained 74-76% of variance in extractable P, 34 - 37% in total P and 64 - 65 % in total N. Total P concentration decreased with both deer access (β = -1.24 to -1.25; SE = 0.14 to 0.18; $p < 0.001$) and earthworm biomass (β = -0.58 to -0.92; SE = 0.14 to 0.15; $p < 0.001$), while extractable P concentration was unaffected by either. Total N concentration was 16% lower in deer access plots (β = -1.17 to -1.19; SE = 0.12 to 0.14; $p < 0.001$). We saw no covariance among soil concentration variables, but total N and extractable P pools were highly correlated ($r=0.91$; $p < 0.001$). In contrast to concentrations, deer access did not impact soil pools. However, earthworm biomass impacted extractable P (β = -0.58; SE = 0.08; $p < 0.001$) and total N (β = -0.42; SE = 0.09; $p < 0.001$) pools.

Table 3-2. Range and mean of soil variables (n = 20 pits).

		Concentrations			Pools (0 - 20 cm)		
Treatment	Dry earthworm biomass (g m-2)	Extractable P (mg kg-1)	Total P (mg kg-1)	Total N (mg kg-1)	Extractable P (mg m-2)	Total P (mg m-2)	Total N (mg m-2)
- Worm - Deer	0.02	1.88	1.07	0.38	1.63	1.00	0.40
-Worm + Deer	0.01	2.21	0.85	0.41	1.50	1.35	10.24
+Worm - Deer	2.65	0.12	0.76	0.46	0.36	1.01	1.73
+Worm + Deer	1.57	0.07	0.54	0.33	0.38	0.85	1.48

Fine roots

Using our models, it was possible to explain <1 -31% of the variability in proportion of fine roots of each species. Earthworm biomass had a positive effect on the proportion of fine roots of *Aquilegia* (Fig 3-3; APPENDIX II.3, II.11, II.19, II.27), and a negative effect on the proportion of fine roots of *Cornus* (Fig 3-5; APPENDIX II.4, II.12, II.20, II.28). In addition to direct earthworm effects, indirect deer and earthworm influences may be mediated through soil pathways. Although this experiment was not designed to test causality among secondary pathways, errors (covariance) of fine root proportion were correlated with total P

concentration in *Actaea* (Fig 3-3; APPENDIX II.2, II.10, II.18, II.26) and *Aquilegia* (Fig 3-4; APPENDIX II.3, II.11, II.19, II.27) and total N concentration in *Prenanthes* (Fig 3-6; APPENDIX II.5, II.13, II.21, II.29). Fine root proportions were correlated with total N pools in *Quercus* and both total N and extractable P pools in *Actaea* and *Cornus* (Fig 3-5; APPENDIX II.4, II.12, II.20, II.28).

AMF

Our models explained 5 – 35% of variance in AMF colonization. Earthworm biomass was associated with lower AMF colonization of *Quercus* roots (Fig 3-7; APPENDIX II.6, II.14, II.22, II.30). Deer did not directly affect mycorrhizal colonization, but had indirect effects through changes to soil variables. In *Aquilegia, Cornus,* and *Prenanthes,* AMF colonization was weakly but significantly positively correlated with changes to total N concentration and pools in *Actaea*, *Aquilegia* and *Cornus* (Figs 3-4, 3-5 & 3-6). To have a converging model for *Actaea* we included extractable P concentration as an additional predictor for AMF colonization, with higher extractable P concentrations being associated with lower AMF colonization (Fig 3-3a,b; APPENDIX II.2, II.10, II.18, II.26). In *Prenanthes*, fine root proportion was positively correlated with mycorrhizal colonization (Fig 3-6). Neither earthworm biomass nor deer significantly impacted absolute vesicle abundance nor vesicle abundance relative to total mycorrhizal colonization in any species (data not shown).

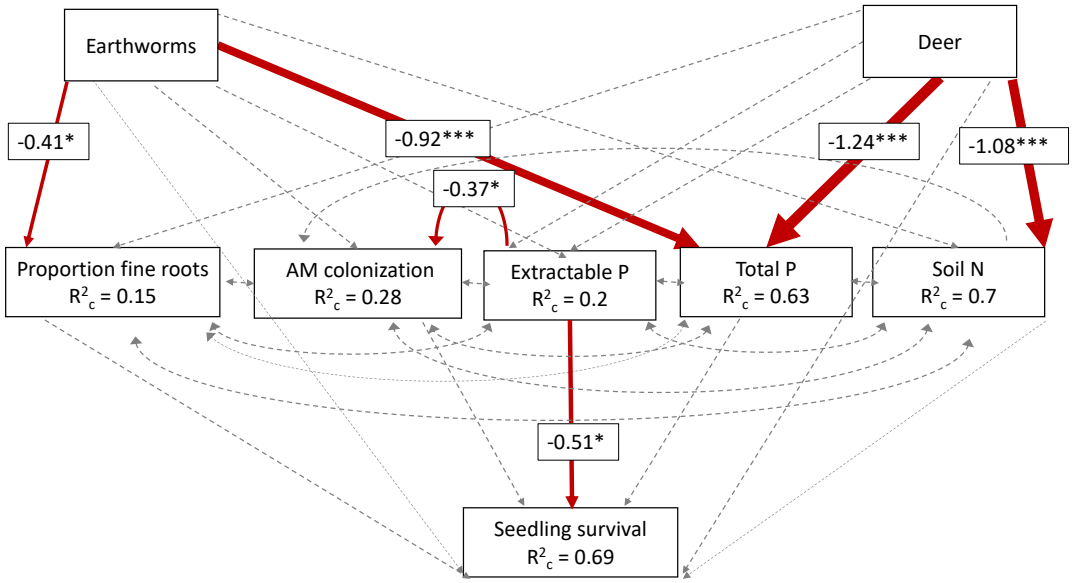
DSE

Our model for the probability of DSE colonization explained 65% of the variance (Table 3-1; Fig 3-8). The only significant predictor of DSE was total N ($\beta = 0.69$ SE = 0.26; $p = 0.007$), which was in turn negatively affected by deer ($\beta = -0.63$; SE = 0.03; $p < 0.001$), suggesting a

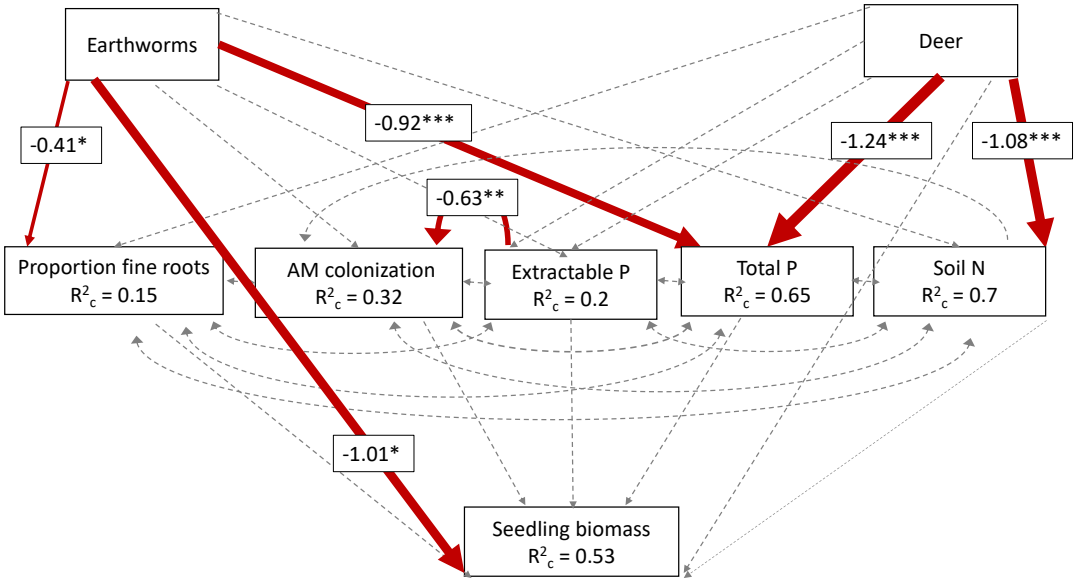
negative indirect effect. Earthworms did not have a direct or indirect effect on DSE, and errors (covariance) were not correlated with fine roots or mycorrhizal colonization.

Actaea

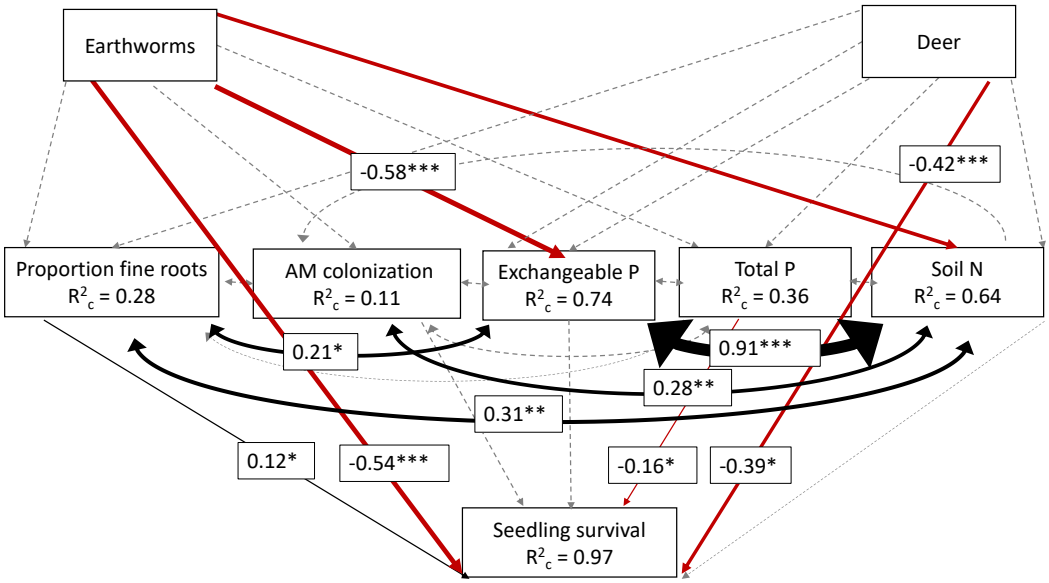
(A) Fisher's C = 7.49; df = 10; p=0.68; AICc = -480.91



(B). Fisher's C = 8.15; p=0.61; AICc = -409.85



(C) Fisher's C = 4.03; p=0.13; AICc = -240.97



(D) Fisher's C = 1.45; p=0.38; AICc = -243.55

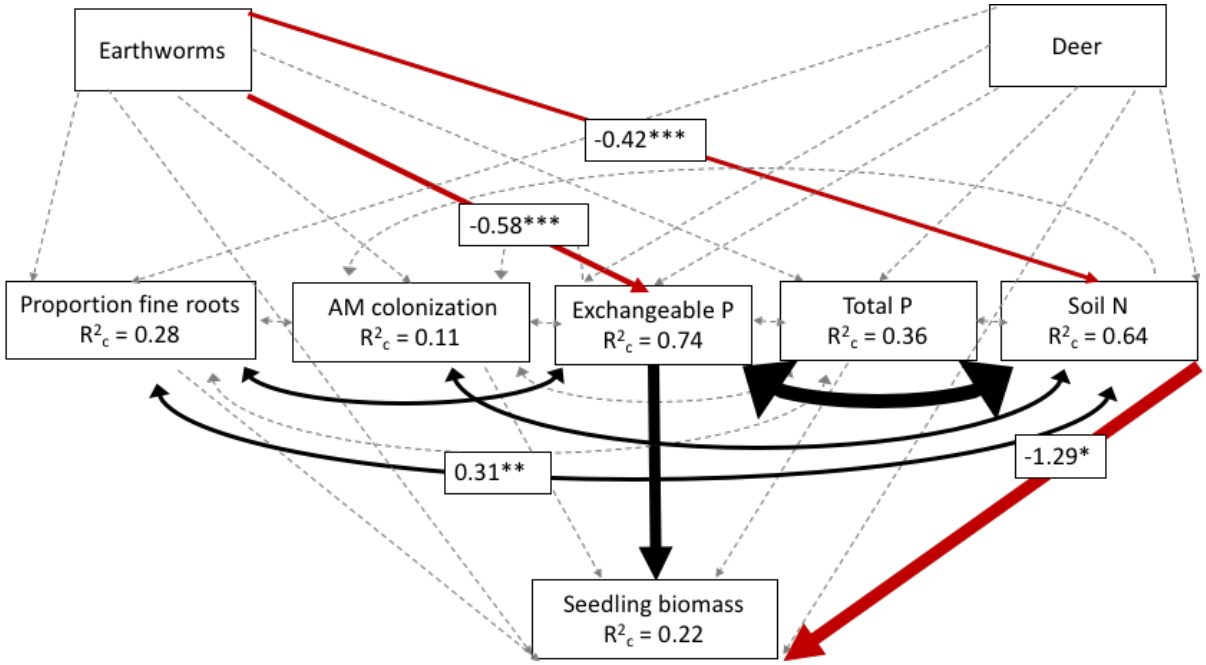
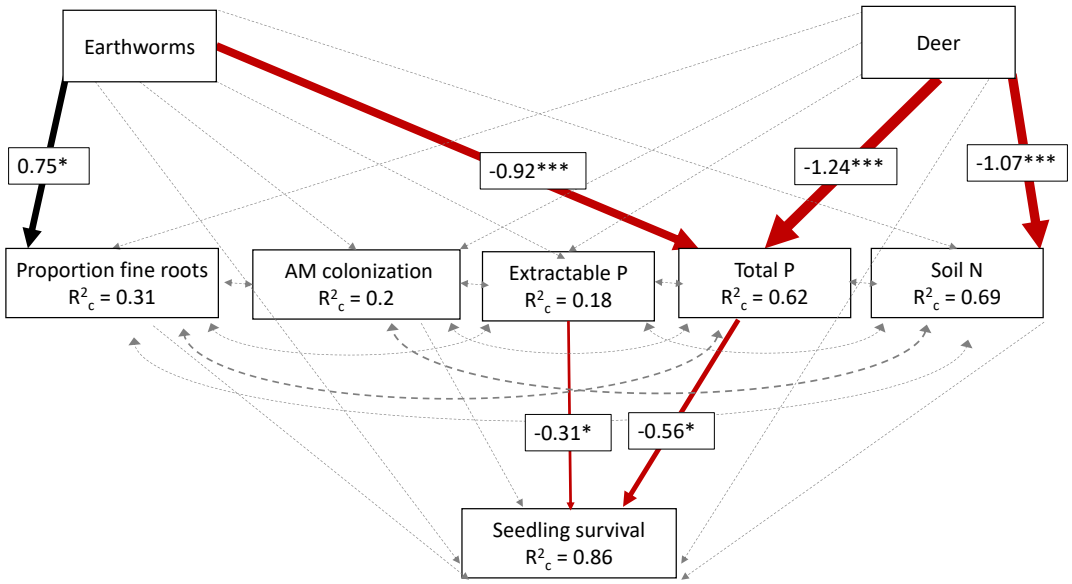


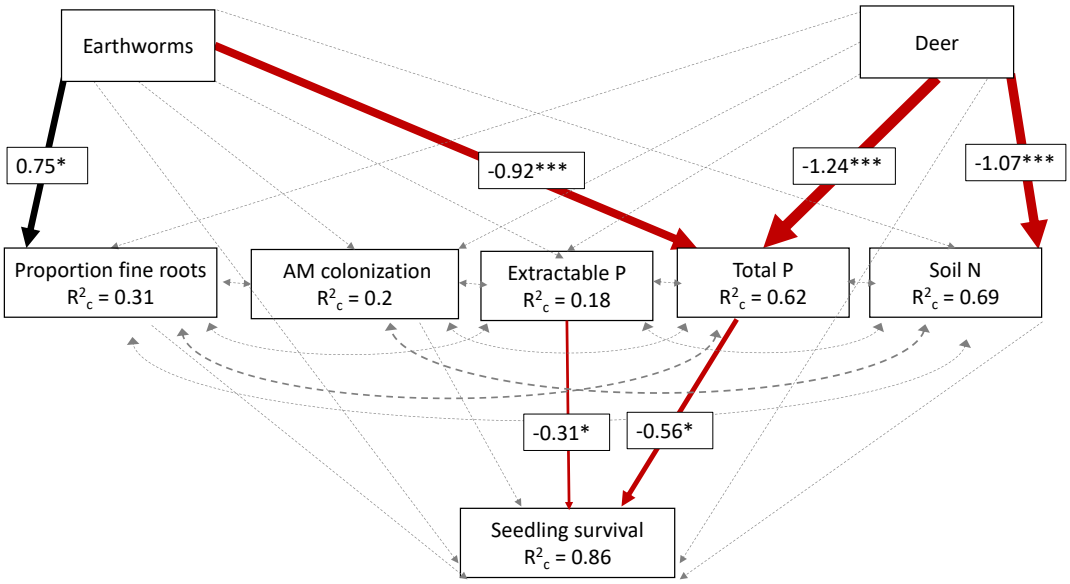
Figure 3-3. Piecewise structural equation models of direct (earthworms and deer) and indirect (AM colonization, proportion of total root length in roots <0.25mm diameter, concentration (A & B) or 20 cm pools (C & D) of total N, extractable P and total soil P) predictors of *Actaea* seedling (A & C) survival and (B & D) dry biomass. Single-headed arrows reflect causative paths and double headed arrows are covariance (correlated errors). Solid red arrows reflect negative paths, solid black arrows reflect negative paths and dotted grey arrows reflect non-significant paths (* P<0.005; ** P<0.01; * P < 0.05). We report the path coefficients in boxes along arrows as standardized effect sizes and conditional R^2 of each exogenous variable.**

Aquilegia

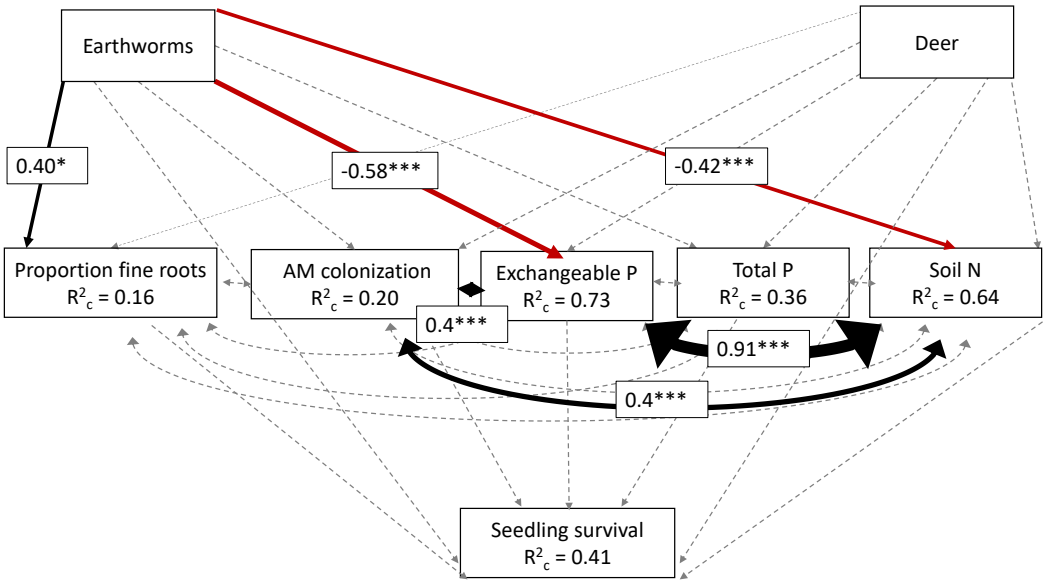
(A) Fisher's C = 8.31; p=0.60; AICc = -52



(B) Fisher's C = 8.31; p=0.60; AICc = -46.5



(C) Fisher's C = 35.33; p = 0; AICc = -41.47



(D) Fisher's C = 6.52; p = 0.37; AICc = -61.48

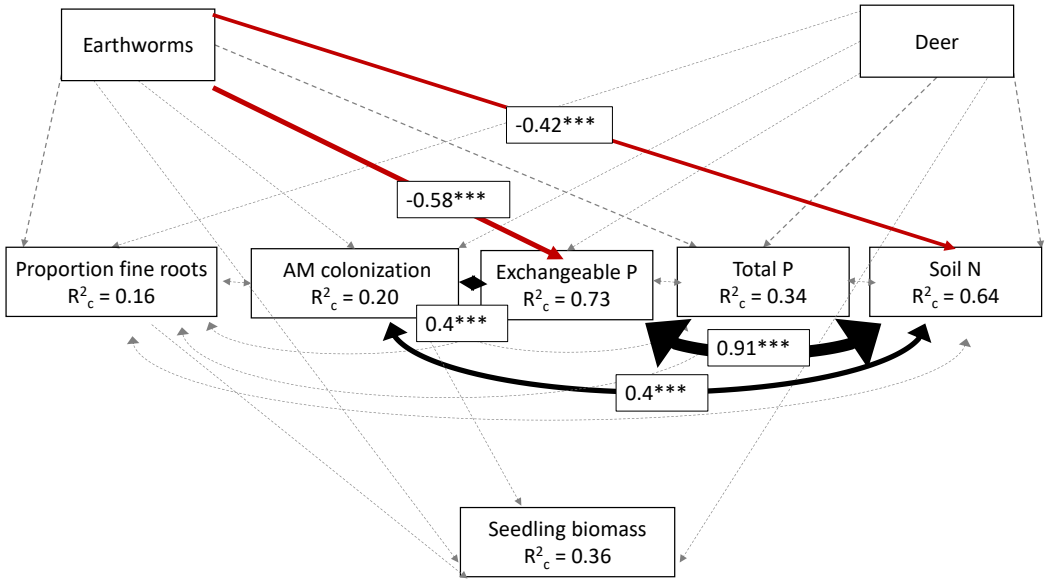
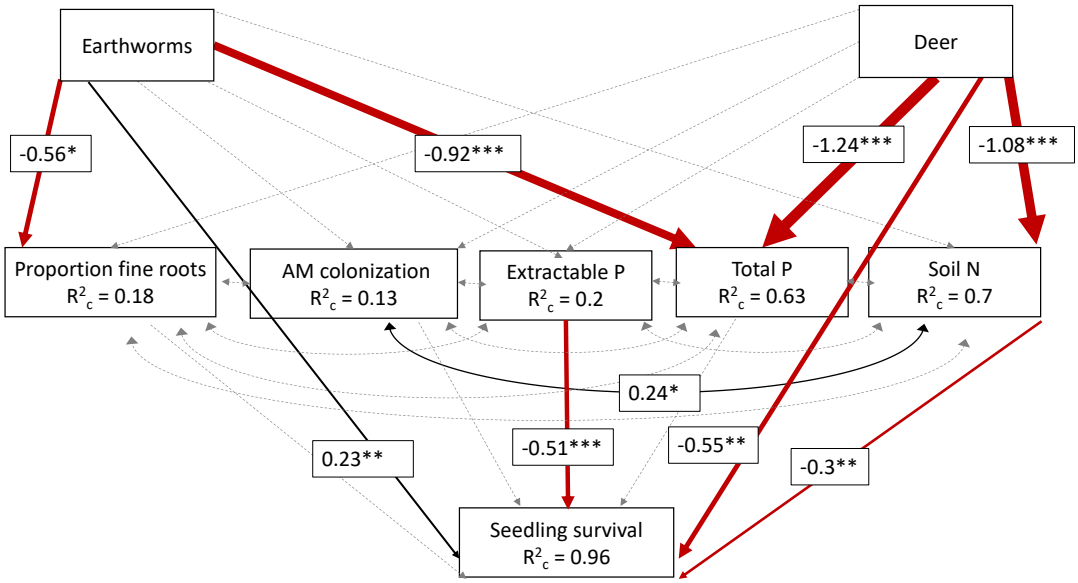


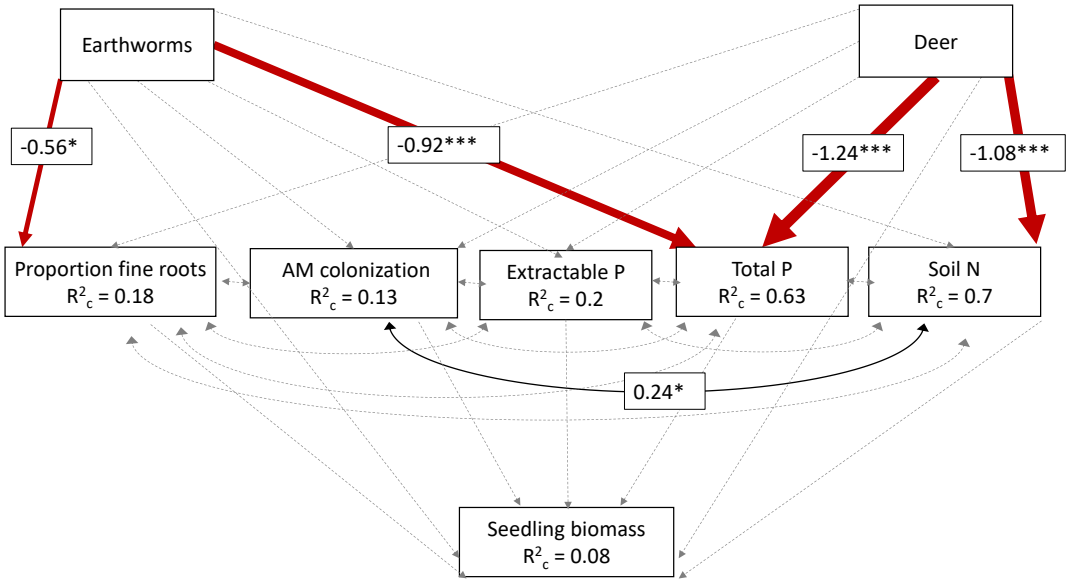
Figure 3-4. Piecewise structural equation models of direct (earthworms and deer) and indirect (AM colonization, proportion of total root length in roots <0.25mm diameter, concentration (A & B) or 20 cm pools (C & D) of total N, extractable P and total soil P) predictors of *Aquilegia* seedling (A & C) survival and (B & D) dry biomass. Single-headed arrows reflect causative paths and double headed arrows are covariance (correlated errors). Solid red arrows reflect negative paths, solid black arrows reflect negative paths and dotted grey arrows reflect non-significant paths (* P<0.005; ** P<0.01; * P < 0.05). We report the path coefficients in boxes along arrows as standardized effect sizes and conditional R^2 of each exogenous variable.**

Cornus

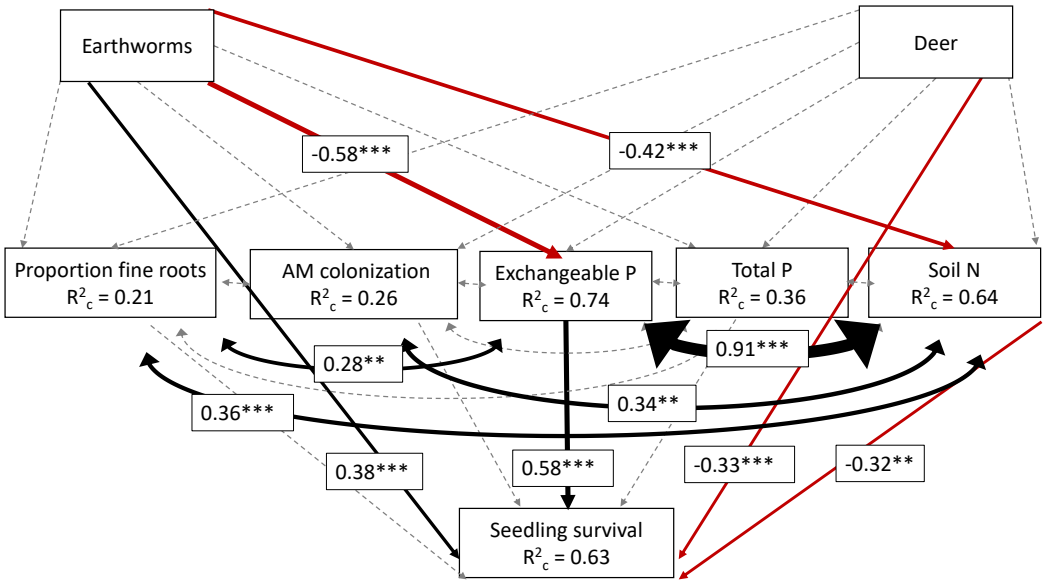
(A) Fisher's C = 13.79; p = 0.31; AICc = 276.21



(B) Fisher's C = 11.25; p = 0.51; AICc = 278.75



(C) Fisher's C = 29.32; p = 0; AICc = -353.48



(D)

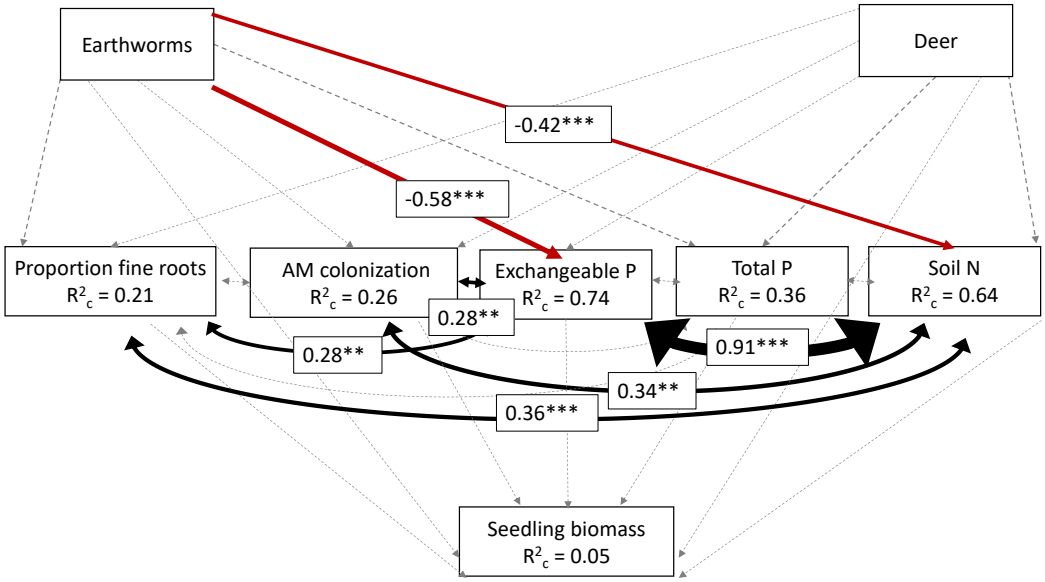
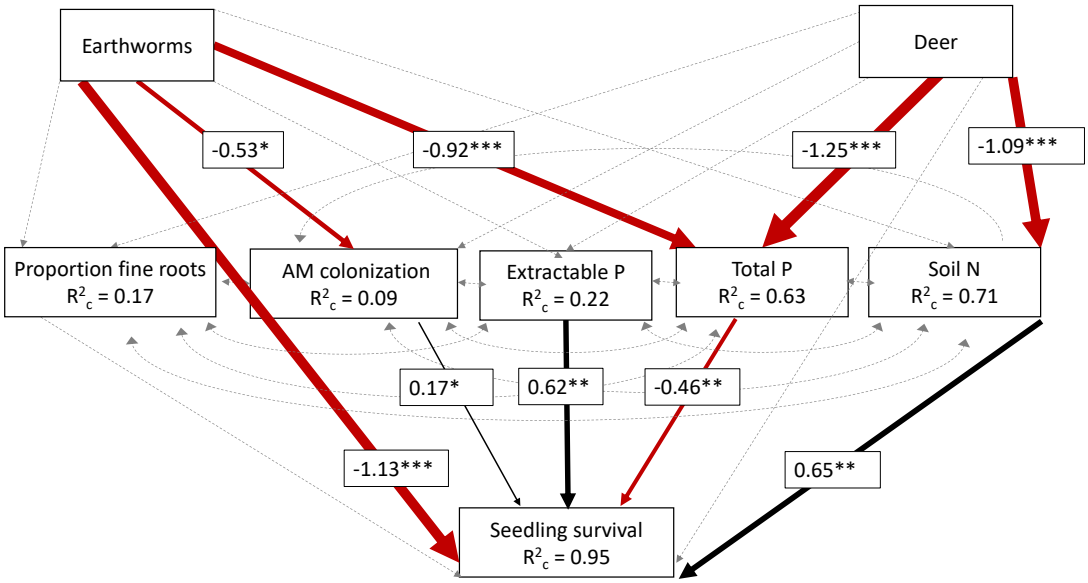


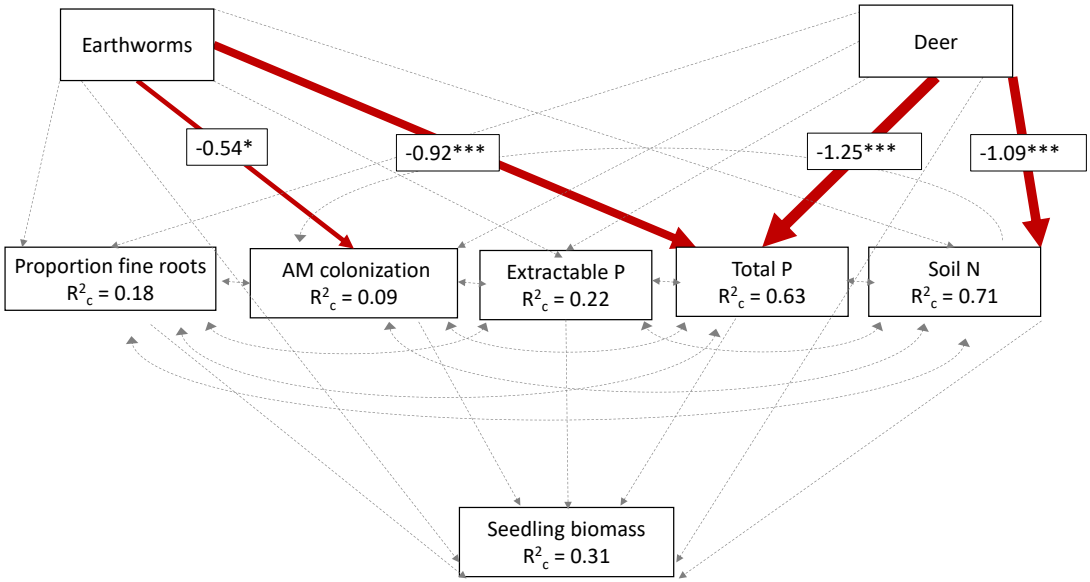
Figure 3-5. Piecewise structural equation models of direct (earthworms and deer) and indirect (AM colonization, proportion of total root length in roots <0.25mm diameter, concentration (A & B) or 20 cm pools (C & D) of total N, extractable P and total soil P) predictors of *Cornus* seedling (A & C) survival and (B & D) dry biomass. Single-headed arrows reflect causative paths and double headed arrows are correlated errors. Solid red arrows reflect negative paths, solid black arrows reflect negative paths and dotted grey arrows reflect non-significant paths (* P<0.005; ** P<0.01; * P < 0.05). We report the path coefficients in boxes along arrows as standardized effect sizes and conditional R^2 of each exogenous variable.**

Quercus

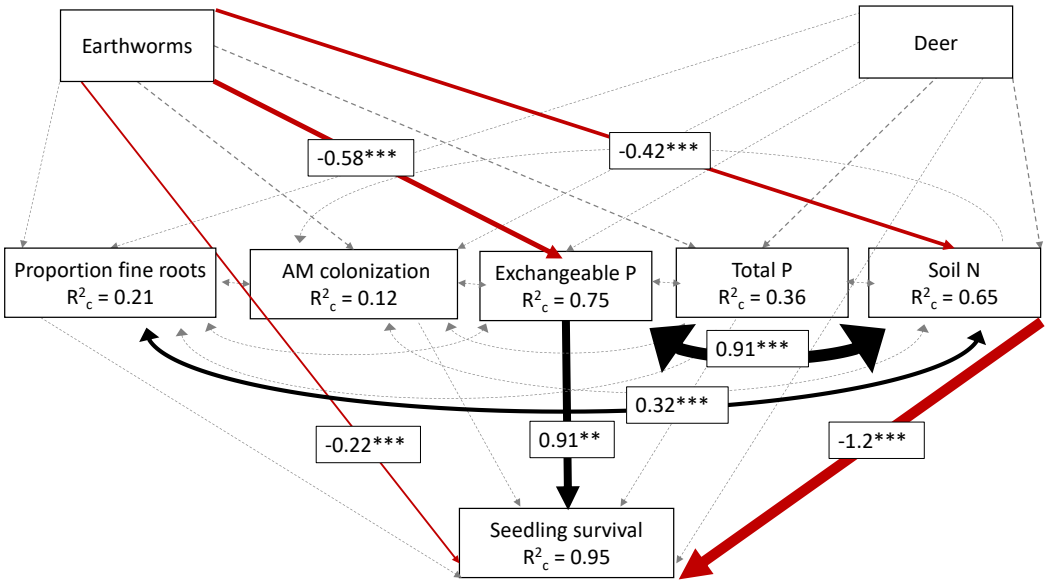
(A) Fisher's C = 10.82; p = 0.37; AICc = -57.4



(B) Fisher's C = 11.72; p = 0.47; AICc = -54.88



(C) Fisher's C = 24.89; p = 0; AICc = 651.89



(D) Fisher's C = 2.19; p = 0.7; AICc = -380.61

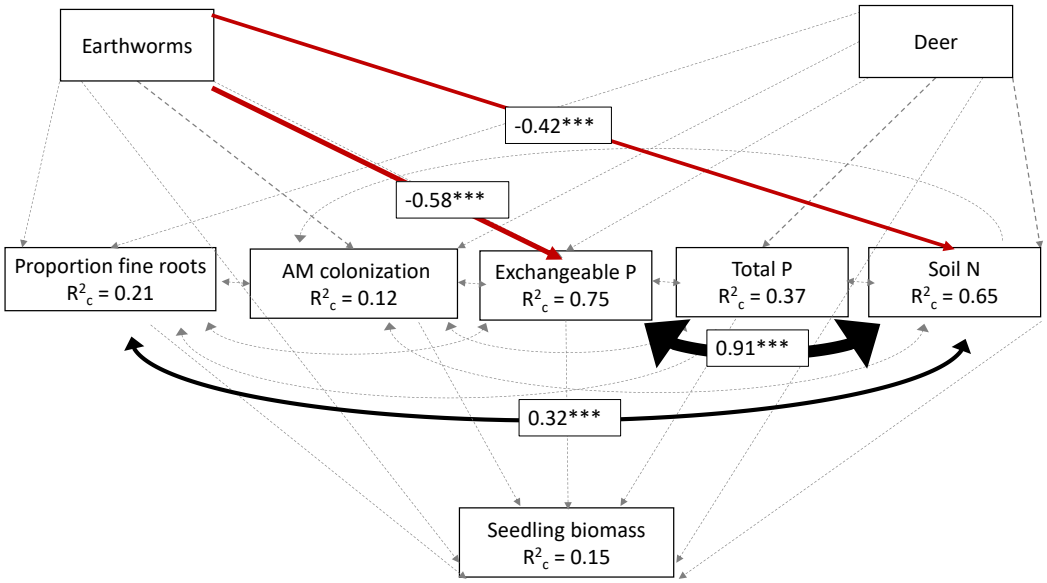
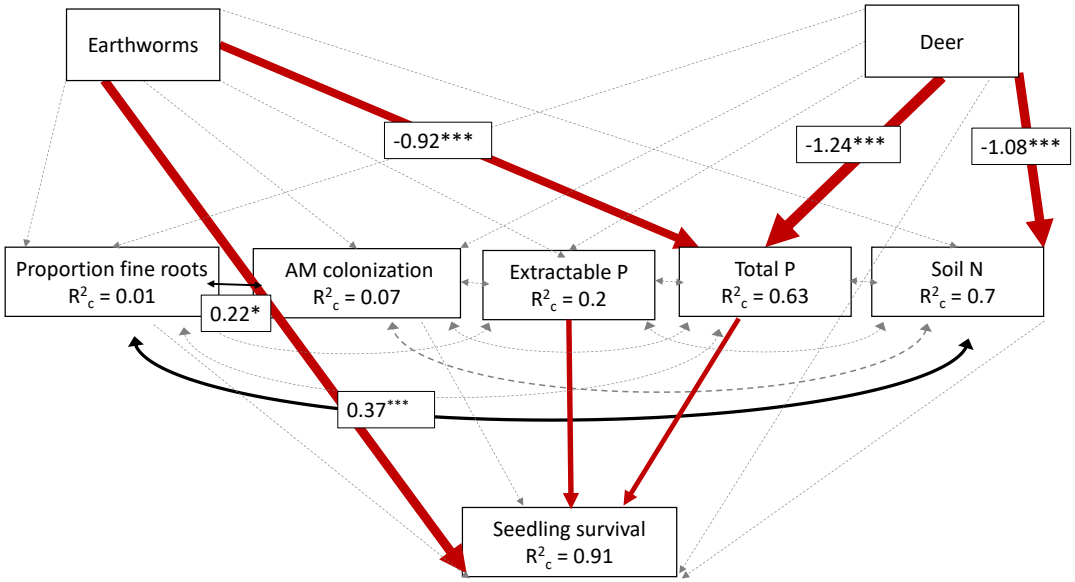


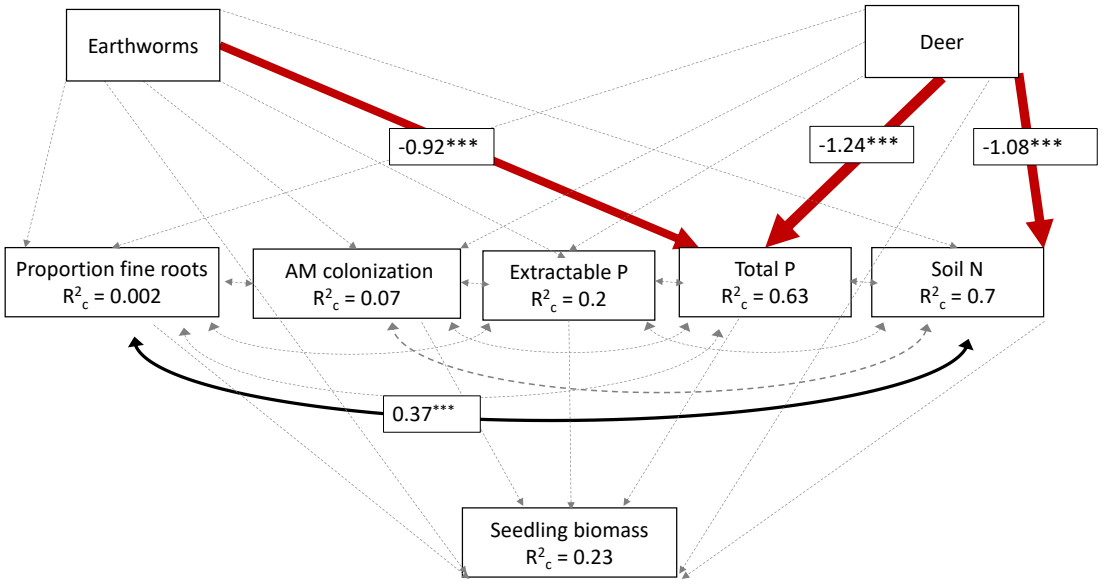
Figure 3-6. Piecewise structural equation models of direct (earthworms and deer) and indirect (AM colonization, proportion of total root length in roots <0.25mm diameter, concentration (A & B) or 20 cm pools (C & D) of total N, extractable P and total soil P) predictors of *Quercus* seedling (A & C) survival and (B & D) dry biomass. Single-headed arrows reflect causative paths and double headed arrows are correlated errors. Solid red arrows reflect negative paths, solid black arrows reflect negative paths and dotted grey arrows reflect non-significant paths (* P<0.005; ** P<0.01; * P < 0.05). We report the path coefficients in boxes along arrows as standardized effect sizes and conditional R^2 of each exogenous variable.**

Prenanthes

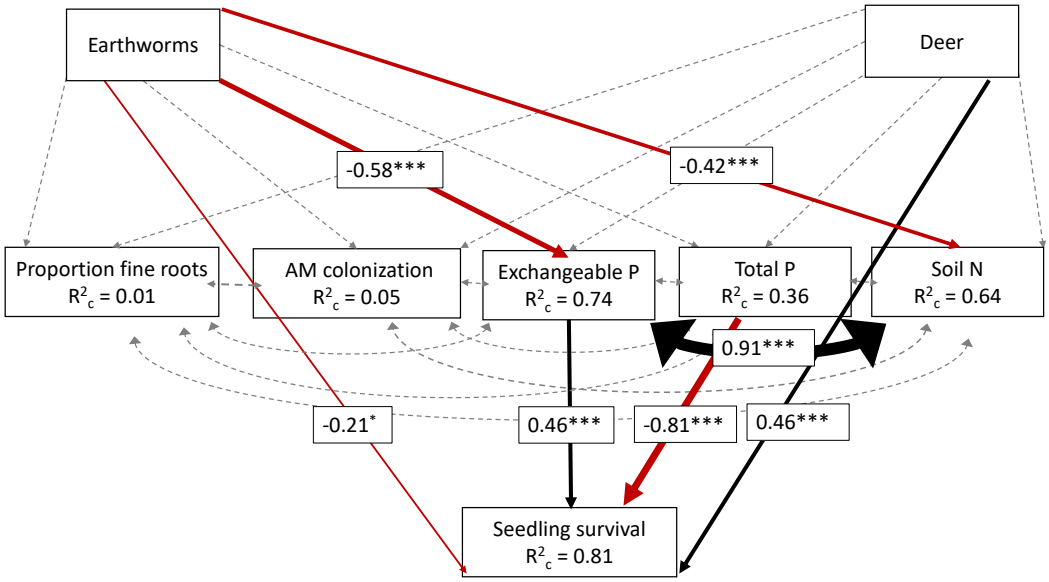
(A) Fisher's C = 5.43; p = 0.86; AICc = -2386.57



(B) Fisher's C = 5.43; p = 0.86; AICc = -1254.57



(C) Fisher's C = 16.46; p = 0.01; AICc = -70.4



(D) Fisher's C = 8.39; p = 0.21; AICc = -78.47

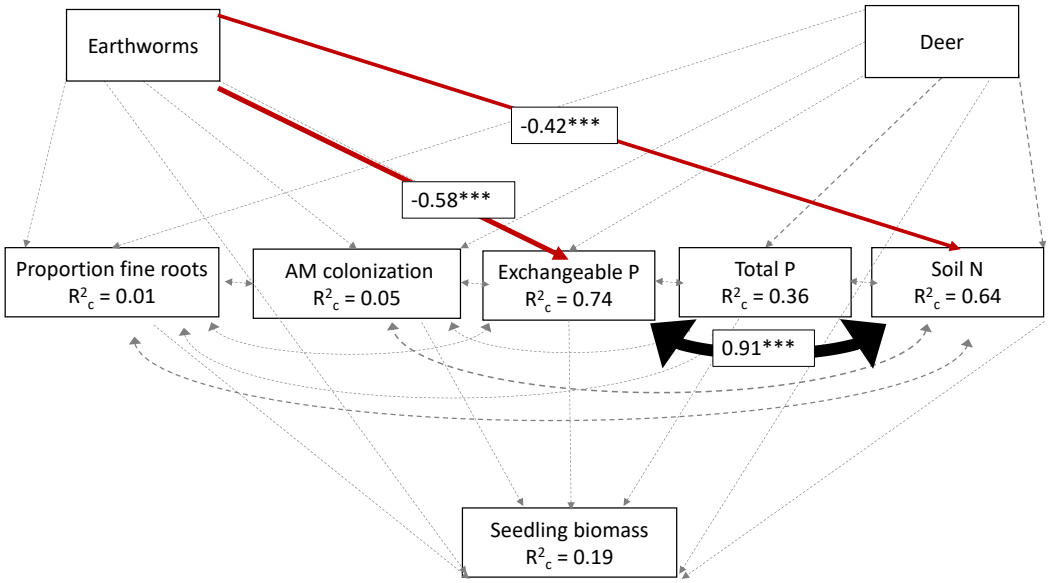


Figure 3-7. Piecewise structural equation models of direct (earthworms and deer) and indirect (AM colonization, proportion of total root length in roots <0.25mm diameter, concentration (A & B) or 20 cm pools (C & D) of total N, extractable P and total soil P) predictors of *Prenanthes* seedling (A & C) survival and (B & D) dry biomass. Single-headed arrows reflect causative paths and double headed arrows are covariance (correlated errors). Solid red arrows reflect negative paths, solid black arrows reflect negative paths and dotted grey arrows reflect non-significant paths (* P<0.005; ** P<0.01; * P < 0.05). We report the path coefficients in boxes along arrows as standardized effect sizes and conditional R^2 of each exogenous variable.**

DISCUSSION

Despite our multifaceted approach, we could not confirm our *a priori* hypotheses for all species. Earthworms and deer had profound effects on soil fertility, decreasing both total N and P concentrations. Deer did not impact pools, however earthworms were associated with smaller extractable P and total N pools. Where fine roots and AMF were affected, the direction of the effect was variable and often did not translate into altered seedling performance. Where we found a direct effect of deer or earthworms on seedling performance, it was often accompanied by multiple indirect effects. Instead of identifying single dominant mechanisms of deer and earthworm impacts on native plant communities, we demonstrate that these stressors impact ecosystems in a variety of ways that affect plant growth and survival. The summation of those negative and positive impacts is different for each species, and ultimately determines how that species will respond to contemporary forest conditions. Despite a lack of agreement across all species, groups of species responded similarly. We observed direct, negative effects of earthworms on *Actaea* biomass and survival of *Quercus* and *Prenanthes,* in support of hypothesis 1. Earthworms had a small direct positive effect on *Cornus* survival, in addition to decreasing pools of total N and exchangeable P, which were respectively associated with lower and higher survival. Deer impacts on seedlings were variable or absent, likely because most of our transplants remained below the 10 - 20cm 'molar zone', buffering them from significant losses in deer access plots (Dobson and Blossey 2015).

Earthworm presence was associated with marked declines in total soil P concentrations as well as extractable P pools. This loss of P from topsoil is consistent with other studies from regions of North America historically devoid of earthworms (Paré and Bernier 1989; Resner et al. 2015), though not universal (Suarez et al. 2004; Hale et al. 2008). Surprisingly, although earthworms eliminated the organic horizon, this process did not enrich

total N concentration in the A horizon, but decreased total N pools. Previous studies have found variable effects of earthworm invasion on forest soil N, with new invasions and pot experiments showing nutrient enrichment associated with decomposition, while forests with higher, established earthworm invasions tend to show an overall loss of nutrients through leaching and erosion (Hale et al. 2008; Watmough and Meadows 2014; Resner et al. 2015; Dobson et al. 2017a).

Excluding deer over a six-year period led to substantial increases in both total N and P concentrations. Murray et al. (2013) found higher levels of available ammonia in deer exclosures, positing that deer increase nitrogen heterogeneity through browsing vegetation and excretion of nitrogenous wastes in small, concentrated patches that vary seasonally. Deer are increasing spatial heterogeneity of nutrients in a landscape could explain why experiments with deer exclosures see variable impacts of deer on soil nutrients (Bressette et al. 2012; Shelton et al. 2014). However, the spatial and temporal extent of our soil sampling design (one pit per plot) does not discount that deer cause net declines in A-horizon N.

Total N and/or P concentrations significantly affected survival of all five species. We saw higher survival of *Quercus* with higher total N and extractable P (Fig 3-7), but most other species had lower survival with increasing N and/or P. This supports the phenomenon emerging from N-deposition studies that plant species richness declines with increasing N, particularly in acidic forests such as these (Simkin et al. 2016). Additionally, plants growing in high nutrient concentrations are more nutrient-rich and thus vulnerable to herbivory (Deniau et al. 2017). *Quercus* may be the exception, where relief from nutrient limitation could explain their higher survival with increasing total N.

Despite these large changes to soil nutrient concentrations associated with deer and earthworms, plant biomass was unaffected by nutrients in all species. However, this does not appear to be due to the plants' ability to maintain consistency despite ecological differences,

but due to the large variability in final plant size (Table 3-1). Further, initial height at planting was not predictive of either biomass or survival in any species. This suggests that variables beyond the scope of this experiment (i.e. light) are more important for these species (Dobson and Blossey 2015).

AMF-mediated effects of earthworms have emerged as an important possible mechanism for the vulnerability of a plant to earthworm invasion (Gundale 2002; Lawrence et al. 2003; McLean et al. 2006; Paudel et al. 2016a). However, we observed a negative impact of earthworms on AMF colonization in *Quercus* only, suggesting that this is not a ubiquitous explanation of the negative effects of plants in all AMF-associated plant species (hypothesis 3). Furthermore, deer presence and earthworm biomass explained < 10% of the variance in AMF colonization of *Quercus*, and AMF colonization did not impact seedling performance. AMF colonization was extremely variable even within species, mirroring results we obtained for biomass, suggesting existence of other important variables that we did not capture. Earthworms may be consuming fungal hyphae or modifying fungal microhabitat, but neither AMF colonization (measure as % of root length with hyphal coils and/or arbuscules) nor mutualism productivity (relative abundance of arbuscules:vesicles) was affected. Similar lack of effects of earthworms on AMF colonization have been found for other single-season studies in both forest and grassland species (Eisenhauer et al. 2009; Wurst and Rillig 2011; Yang et al. 2015). Cameron et al. (2012) suggest that AMF colonization may recover relatively quickly after earthworm disturbance, with earthworms acting as vectors for AMF spores. Indeed, a study at a nearby forest, Dempsey and others (2013) observed increased AMF phospholipid-derived fatty acids in surface mineral soil, attributing the increase to earthworms spreading AMF propagules through the soil (Gange 1993). Taken together, this could suggest that earthworm-associated declines in mycorrhizal fungi may be associated with early stages of invasion, and AMF is able to recolonize.

However, plant diversity will not recover if AMF-obligate plant species are lost from the seedbank before the fungi can recolonize.

Deer did not directly affect AMF colonization in any species, rejecting the explanation that non-consumptive effects of deer on unpalatable species occurs through disruption of AMF-plant mutualisms. Our results agree with Shelton et al. (2014), who saw no change in AMF colonization after two to seven years of deer exclusion in an Indiana forest. In contrast, Rossow et al. (1997) found that moose decrease ectomycorrhizal colonization in a taiga forest, suggesting that we cannot generalize to effects of ungulates in other ecosystems.

Our data present a snapshot of mycorrhizal response to deer and earthworms; however mycorrhizal activity is variable between and within a growing season (Brundrett and Kendrick 1988; Brundrett et al. 1996). Because earthworms alter temperature, moisture and nutrient properties of soils, their influence on seasonal mycorrhizal fluctuations (such as spring recolonization) may provide more detailed information about consequences to fungal communities (Larson et al. 2010; Eisenhauer et al. 2012). Finally, although we planted bare-root transplants, we grew them initially in potting soil, which may be colonized differently than seeds falling from adults with their own unique microbiome (Bloomberg 1966; Links et al. 2014). Taken together, this suggests that earthworm effects are different for different AMF-plant mutualisms, and we should limit broad generalizations of earthworm effects on AMF.

In response to declining soil P concentrations resulting from high deer populations and earthworm invasions, we would expect to see increased mycorrhizal colonization (Bressette et al. 2012; Kluber et al. 2012). However, we only observed correlated errors between mycorrhizal colonization and extractable P in *Actaea* (Fig 3-3). Therefore, even where deer and earthworms are not directly affecting mycorrhizae, they may stress these

relationships such that AMF do not respond to P declines following earthworm invasion. Ultimately, a plant's ability to respond to nutrient concentrations may determine its response to stressors such as deer and earthworms. Prolonged earthworm invasion may deplete extractable soil P, with consequences for species such as *Actaea* (Resner et al. 2015; Dobson et al. 2017a). For other species in which we did not see a relationship between extractable P and mycorrhizal colonization, it is possible that P is so limiting in all of our study areas that AMF-plant mutualisms are ubiquitous or that plants are responding to differences on a smaller scale of P differences than our experiment captured. The former is supported by a previous study in the same plots showing the majority of *A. saccharum* seedlings were below the foliar threshold for P-limitation (Dobson et al. 2017a).

Surprisingly, total N was more correlated with AMF colonization than either total or extractable P. For both *Cornus* and *Prenanthes*, mycorrhizal colonization was positively correlated with total N. A robust AMF community may support higher quality (higher N) litter. Alternatively, N may be a primary limiting nutrient for AMF, and although they use inorganic N, they proliferate in patches with high SOM (Hodge and Fitter 2010). And while AMF can effectively mine N for their own tissue, this does not always translate to enhanced plant growth (Hodge and Fitter 2010; Yang et al. 2015).

DSE were not excluded nor facilitated by AMF, but appear to respond to the same forces. While not directly affected by deer or earthworms as we expected, colonization by both DSE and AMF was higher with increased total N. DSE associations with plants range from mutualistic to parasitic, depending on growing conditions, host plant species and fungal species or genotype (Jumpponen 2001). Nitrogen can influence how DSE interact with plants, with higher soil N causing DSE to affect plant growth and P acquisition similar to mycorrhiza (Jumpponen et al. 1998). Therefore, in addition to influencing DSE colonization

through changes to soil nutrient pools and concentrations, deer and earthworms may modify the activity of DSE.

Dark Septate Endophyte

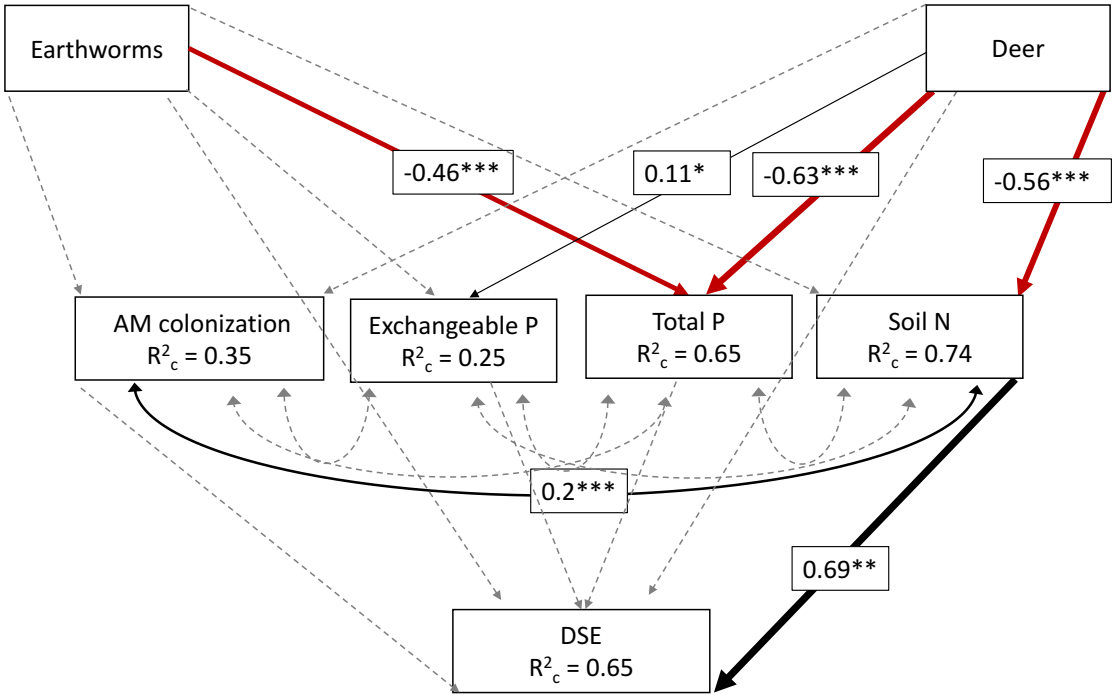


Figure 3-8. Piecewise structural equation models of direct (earthworms and deer) and indirect (AM colonization, proportion of total root length in roots <0.25mm diameter, total N, concentration of total soil P) predictors of dark septate endophyte (DSE) colonization of roots. Single-headed arrows reflect causative paths and double headed arrows are covariance (correlated errors). Solid red arrows reflect negative paths, solid black arrows reflect negative paths and dotted grey arrows reflect non-significant paths (* P<0.005; ** P<0.01; * P < 0.05). We report the path coefficients in boxes along arrows as standardized effect sizes and conditional R^2 of each exogenous variable.**

Three of our five study species showed changes to root architecture in response to earthworms. A lower proportion of roots in *Actaea* and *Cornus* were fine (<0.25 mm) in plants growing with earthworms, while the opposite was true of *Aquilegia*. While we do not have sufficient phylogenetic replication to confirm the role of life history, this result supports the hypothesis that the response of native plants to invasive earthworms may depend on traits. It is possible that slow-growing species such as *Actaea* and *Cornus* respond differently to the physical disturbance of earthworms, or that earthworms are consuming more fine roots

than the plants can replace (Fisk et al. 2004a; Gilbert et al. 2014; Paudel et al. 2016a). Fine root growth of fast-growing species such as *Prenanthes* may be stimulated either by earthworm herbivory or in response to rapid mineralization of soil nutrients. In contrast, woody species have less plasticity to respond to stressors with alternative root architecture strategies (Liu et al. 2015). Notably, changes to fine roots did not have an effect on either seedling survival or plant biomass, rejecting hypothesis (4) that this is a major pathway by which deer and earthworms influence growth and survival of understory plant species. Changes in fine roots that we observed may represent plastic responses, where plants are able to buffer themselves from spatial, temporal and seasonal changes to their habitat.

While this study focuses on forests of northeastern North America, both deer and invasive earthworm populations are increasing worldwide. Japan, Europe, and several temperate islands have seen extremely successful introductions of deer, and existing populations have increased dramatically as land use shifts and predation is limited (Seki et al. 2014; Shelton et al. 2014). A small number of invasive earthworm species have spread globally through tropical and temperate habitats, displacing native species or invading habitats previously devoid of earthworms (Hendrix et al. 2008; Eisenhauer et al. 2012). These influences are not static over time, and our results differ from deer exclosures and earthworm invasions occurring over different time scales (Hale et al. 2008; Bressette et al. 2012). It will be increasingly important to understand how these forces influence above and belowground communities globally and over time.

CONCLUSIONS

Understanding the implications of novel stressors can be a challenging prospect when their impacts are wide reaching and difficult to disentangle. Despite our design that was able to separate direct and indirect effects of individual stresses, we did not identify one clear belowground pathway through which deer and earthworms affect seedlings. Earthworms and deer had large impacts on soil nutrients and fine roots, but this did not always translate to diminished seedling performance.

WORKS CITED

Augustine DJ, McNaughton SJ. 1998. Ungulate effects on the functional species composition of plant communities: herbivore selectivity and plant tolerance. J Wildl Manage:1165–83.

Bardgett RD, Putten WH Van Der. 2014. Belowground biodiversity and ecosystem functioning. Nature 515:505–11.

Baylis GTS. 1970. Root hairs and phycomycetous mycorrhizas in phosphorus deficient soil. Plant Soil 716:713–6.

Blanke V, Renker C, Wagner M, Füllner K, Held M, Kuhn AJ, Buscot F, Blanke V. 1991. Nitrogen supply affects arbuscular mycorrhizal colonization of *Artemisia vulgaris* in a phosphate-polluted field site. New Phyto 166:981–92.

Bloomberg WJ. 1966. The occurrence of endophytic fungi in Douglas fir seedlings and seed. Can J Bot 44:413–20.

Bohlen PJ, Groffman PM, Fahey TJ, Fisk MC, Suarez E, Pelletier DM, Fahey RT. 2004a. Ecosystem consequences of exotic earthworm invasion of north temperate forests. Ecosystems 7:1–12.

Bohlen PJ, Pelletier DM, Groffman PM, Fahey TJ, Fisk MC. 2004b. Influence of earthworm invasion on redistribution and retention of soil carbon and nitrogen in northern temperate forests. Ecosystems 7:13–27.

Bolan NS. 1991. A critical review on the role of mycorrhizal fungi in the uptake of phosphorus by plants. Plant Soil 189–207:189–207.

Bonkowski M, Griffiths BS, Ritz K. 2000. Food preferences of earthworms for soil fungi. Pedobiologia (Jena) 44:666–76.

Bonneau L, Wipf D, Pauly N, Truong H. 2013. Combined phosphate and nitrogen limitation generates a nutrient stress transcriptome favorable for arbuscular mycorrhizal symbiosis

in *Medicago truncatula*.

Bressette JW, Beck H, Beauchamp VB. 2012. Beyond the browse line: complex cascade effects mediated by white-tailed deer. Oikos 121:1749–60.

Brundrett M. 2004. Diversity and classification of mycorrhizal associations. Biol Rev 79:473–95.

Brundrett MC. 2009. Mycorrhizal associations and other means of nutrition of vascular plants: Understanding the global diversity of host plants by resolving conflicting information and developing reliable means of diagnosis. Plant Soil 320:37–77.

Brundrett MC, Boughear N, Dell B, Grove T, Malajczuk N. 1996. Working with mycorrhizas in forestry and agriculture. Canberra, Australia: Australian Centre for International Agricultural Research

Brundrett MC, Kendrick B. 1988. The mycorrhizal status, root anatomy, and phenology of plants in a sugar maple forest. Can J Bot 66:1153–73.

Burtelow AE, Bohlen PJ, Groffman PM. 1998. Influence of exotic earthworm invasion on soil organic matter, microbial biomass and denitrification potential in forest soils of the northeastern United States. Appl Soil Ecol 9:197–202.

Cameron EK, Zabrodski MW, Karst J, Bayne EM. 2012. Non-native earthworm influences on ectomycorrhizal colonization and growth of white spruce. Ecoscience 19:29–37.

Cappellazzo G, Lanfranco L, Bonfante P. 2007. A limiting source of organic nitrogen induces specific transcriptional responses in the extraradical structures of the endomycorrhizal fungus Glomus intraradices. Curr Genet 51:59–70.

Chapman HD. 1965. Cation-exchange capacity 1. In: Norman AG, editor. Methods of soil analysis. Part 2. Chemical and microbiological properties. Agronomy Monograph SV - 9.2. Madison, WI: American Society of Agronomy, Soil Science Society of America. pp 891–

Chaudhary VB, Bowker MA, O'Dell TE, Grace JB, Redman AE, Rilling MC, Johnson NC. 2009. Untangling the biological contributions to soil stability in semiarid shrublands. Ecol Appl 19:110–22.

Chimungu JG, Brown KM, Lynch JP. 2014. Large root cortical cell size improves drought tolerance in maize. Plant Physiol 166:2166–78.

Ciesielski H, Sterckeman T, Santerne M, Willery J. 1997. A comparison between three methods for the determination of cation exchange capacity and exchangeable cations in soils. agronomie 17:9–16.

Côté IM, Darling ES, Brown CJ. 2016. Interactions among ecosystem stressors and their importance in conservation. Proc R Soc B Biol Sci 283:20152592.

Côté SD, Rooney TP, Tremblay J-P, Dussault C, Waller DM. 2004. Ecological impacts of deer overabundance. Annu Rev Ecol Evol Syst 35:113–47.

Dávalos A, Nuzzo V, Blossey B. 2014. Demographic responses of rare forest plants to multiple stressors: The role of deer, invasive species and nutrients. J Ecol 102:1222–33.

Dávalos A, Nuzzo V, Blossey B. 2015a. Forest Ecology and Management Single and interactive effects of deer and earthworms on non-native plants. For Ecol Manage 351:28–35.

Dávalos A, Simpson E, Nuzzo V, Blossey B. 2015b. Non-consumptive effects of native deer on introduced earthworm abundance. Ecosystems 18:1029–42.

Dempsey MA, Fisk MC, Fahey TJ. 2011. Earthworms increase the ratio of bacteria to fungi in northern hardwood forest soils, primarily by eliminating the organic horizon. Soil Biol Biochem 43:2135–41.

Dempsey MA, Fisk MC, Yavitt JB, Fahey TJ, Balser TC. 2013. Exotic earthworms alter soil microbial community composition and function. Soil Biol Biochem 67:263–70.

Deniau M, Jung V, Le Lann C, Kellner H, Bechade B, Morra T, Prinzing A. 2017. Janzen-

Connell patterns can be induced by fungal-driven decomposition and offset by ectomycorrhizal fungi accumulated under a closely related canopy. Funct Ecol:0–2.

Dickie IA, Koide RT, Fayish AC, Koide RT. 2001. Vesicular – arbuscular mycorrhizal infection of Quercus rubra seedlings. :257–64.

Dickson S. 2004. The Arum-Paris continuum of mycorrhizal symbioses. New Phytol 163:187–200.

Didham RK, Tylianakis JM, Hutchison MA, Ewers RM, Gemmell NJ. 2005. Are invasive species the drivers of ecological change? Trends Ecol Evol 20:470–4.

Dobson A, Blossey B. 2015. Earthworm invasion, white-tailed deer and seedling establishment in deciduous forests of north-eastern North America. J Ecol 103:153–64.

Dobson AM, Blossey B, Richardson JB. 2017. Invasive earthworms change nutrient availability and uptake by forest understory plants. 421:175-190.

Doughty CE. 2017. Herbivores increase the global availability of nutrients over millions of years. Nat Ecol Evol 1:1820–7.

Eisenhauer N. 2010. The action of an animal ecosystem engineer: Identification of the main mechanisms of earthworm impacts on soil microarthropods. Pedobiologia (Jena) 53:343–52.

Eisenhauer N. 2017. Consumers control carbon. Nat Ecol Evol 1:1596–7.

Eisenhauer N, Bowker M a., Grace JB, Powell JR. 2015. From patterns to causal understanding: Structural equation modeling (SEM) in soil ecology. Pedobiologia (Jena) 58:65–72.

Eisenhauer N, Fisichelli NA, Frelich LE, Reich PB. 2012. Interactive effects of global warming and ‘global worming’ on the initial establishment of native and exotic herbaceous plant species. Oikos 121:1121–33.

Eisenhauer N, Konig S, Sabais ACW, Renker C, Buscot F, Scheu S. 2009. Soil Biology &

Biochemistry Impacts of earthworms and arbuscular mycorrhizal fungi (*Glomus intraradices*) on plant performance are not interrelated. Soil Biol Biochem 41:561–7.

Fahey TJ, Yavitt JB, Sherman RE, Maerz JC, Groffman PM, Fisk MC, Bohlen PJ. 2013. Earthworm effects on the incorporation of litter C and N into soil organic matter in a sugar maple forest. Ecol Appl 23:1185–201.

Fisichelli N, Frelich L, Reich P, Eisenhauer N. 2013. Linking direct and indirect pathways mediating earthworms, deer, and understory composition in Great Lakes forests. Biol Invasions 15:1057–66.

Fisk MC, Fahey TJ, Groffman PM, Bohlen PJ. 2004. Earthworm invasion, fine-root distributions, and soil respiration in north temperate forests. Ecosystems 7:55–62.

Fletcher DJ, Shipley LA, McShea WJ, Shumway DL. 2001. Wildlife herbivory and rare plants: the effects of white-tailed deer, rodents, and insects on growth and survival of Turk's cap lily. Biol Conserv 101:229–38.

Francis GS, Fraser PM. 1998. The effects of three earthworm species on soil macroporosity and hydraulic conductivity. Appl Soil Ecol 10:11–9.

Frelich LE, Hale CM, Scheu S, Holdsworth AR, Heneghan L, Bohlen PJ, Reich PB. 2006. Earthworm invasion into previously earthworm-free temperate and boreal forests. Biol Invasions 8:1235–45.

Frerker K, Sabo A, Waller D. 2014. Long-term regional shifts in plant community composition are largely explained by local deer impact experiments. PLoS One 9:1–17.

Gange AC. 1993. Translocation of mycorrhizal fungi by earthworms during early succession. Soil Biol Biochem 25:1021–6.

Gilbert KJ, Fahey TJ, Maerz JC, Sherman RE, Bohlen P, Dombroskie JJ, Groffman PM, Yavitt JB. 2014. Exploring carbon flow through the root channel in a temperate forest soil food web. Soil Biol Biochem 76:45–52.

Grace JB. 2006. Structural Equation Modeling and Natural Systems. Cambridge, UK: Cambridge University Press

Grarock K, Tidemann CR, Wood JT, Lindenmayer DB. 2014. Are invasive species drivers of native species decline or passengers of habitat modification? A case study of the impact of the common myna (*Acridotheres tristis*) on Australian bird species. Austral Ecol 39:106–14.

Gundale MJ. 2002. Influence of exotic earthworms on the soil organic horizon and the rare fern *Botrychium mormo*. Conserv Biol 16:1555–61.

Hale C, Frelich L, Reich P, Pastor J. 2005. Effects of European earthworm invasion on soil characteristics in northern hardwood forests of Minnesota, USA. Ecosystems 8:911–27.

Hale CM, Frelich LE, Reich PB. 2006. Changes in cold-temperate hardwood forest understory plant communities in response to invasion by European earthworms. Ecology 87:1637–49.

Hale CM, Frelich LE, Reich PB, Pastor J. 2008. Exotic earthworm effects on hardwood forest floor, nutrient availability and native plants: A mesocosm study. Oecologia 155:509–18.

Halls LK. 1984. White-tailed deer: ecology and management. Harrisburg, Pennsylvania, USA: Stackpole Books

Heckel CD, Bourg NA, McShea WJ, Kalisz S. 2010. Nonconsumptive effects of a generalist ungulate herbivore drive decline of unpalatable forest herbs. Ecology 91:319–26.

Hendrix PF, Callaham M a., Drake JM, Huang C-Y, James SW, Snyder B a., Zhang W. 2008. Pandora's Box contained bait: The global problem of introduced earthworms *. Annu Rev Ecol Evol Syst 39:593–613.

Hodge A, Fitter AH. 2010. Substantial nitrogen acquisition by arbuscular mycorrhizal fungi from organic material has implications for N cycling.107:13754-13759.

James SW. 1995. Systematics, biogeography, and ecology of nearctic earthworms from eastern, central, southern and southwestern United States. In: Hendrix PF, editor. Earthworm ecology and biogeography in North America. Lewis. pp 29–52.

Jing X, Sanders NJ, Shi Y, Chu H, Classen AT, Zhao K, Chen L, Shi Y, Jiang YJ, He J-S. 2015. The links between ecosystem multifunctionality and above- and belowground biodiversity are mediated. Nat Commun 6:1–8.

Johansen A, Jakobsen I, Jensen ES. 1994. Hyphal N transport by a vesicular-arbuscular mycorrhizal fungus associated with cucumber grown at three nitrogen levels. 160:1–9.

Johnson AS, Hale PE, Ford WM, Wentworth JM, French JR, Anderson OF, Pullen GB, Sydneyjohnson A, Hale PE, Anderson OF, Pullen GB. 1995. White-tailed deer foraging in relation to successional stage, overstory type and management of Southern Appalachian forests. Am Midl Nat 133:18–35.

Jumpponen A. 2001. Dark septate endophytes - Are they mycorrhizal? Mycorrhiza 11:207–11.

Jumpponen A, Mattson KG, Trappe JM. 1998. Mycorrhizal functioning of Phialocephala fortinii with Pinus contorta on glacier forefront soil : Interactions with soil nitrogen and ... Mycorrhizal functioning of *Phialocephala fortinii* with *Pinus contorta* on glacier forefront soil : interactions with s. Mycorrhiza 7:261–5.

Kirchberger J, Eisenhauer N, Weisser WW, Türke M. 2015. Experimental evaluation of herbivory on live plant seedlings by the earthworm *Lumbricus terrestris* in the presence and absence of soil surface litter. PLoS One 10:e0123465.

Kluber LA, Carrino-kyker SR, Coyle KP, Deforest JL, Hewins CR, Shaw AN, Smemo KA, Burke DJ. 2012. Mycorrhizal response to experimental pH and P manipulation in acidic hardwood forests. PLoS One 7:1–10.

Kramer-Walter KR, Bellingham PJ, Millar TR, Smissen RD, Richardson SJ, Laughlin DC.

2016. Root traits are multidimensional: specific root length is independent from root tissue density and the plant economic spectrum. J Ecol 104:1311–1313.

Kuebbing SE, Classen AT, Sanders NJ, Simberloff D. 2015. Above- and below-ground effects of plant diversity depend on species origin: An experimental test with multiple invaders. New Phytol 208:727–35.

Laliberte E, Lambers H, Burgess TI, Wright SJ. 2015. Phosphorus limitation, soil-borne pathogens and the coexistence of plant species in hyperdiverse forests and shrublands. New Phytol 206:507–21.

Larson ER, Kipfmueller KF, Hale CM, Frelich LE, Reich PB. 2010. Tree rings detect earthworm invasions and their effects in northern Hardwood forests. Biol Invasions 12:1053–66.

Lawrence B, Fisk MC, Fahey TJ, Suarez ER. 2003. Influence of nonnative earthworms on mycorrhizal colonization of sugar maple (*Acer saccharum*). New Phytol 157:145–53.

Lefcheck JS. 2015. PIECEWISE SEM : Piecewise structural equation modelling in R for ecology , evolution , and systematics. 7:573-579.

Links MG, Demeke T, Gr T, Hill JE, Hemmingsen SM, Dumonceaux TJ. 2014. Simultaneous profiling of seed-associated bacteria and fungi reveals antagonistic interactions between microorganisms within a shared epiphytic microbiome on *Triticum* and *Brassica* seeds.

Liu B, Li H, Zhu B, Koide RT, Eissenstat DM, Guo D. 2015. Complementarity in nutrient foraging strategies of absorptive fine roots and arbuscular mycorrhizal fungi across 14 coexisting subtropical tree species. :125–36.

Luke T, Kohout P, Vohník M. 2015. The potential of dark septate endophytes to form root symbioses with ectomycorrhizal and ericoid mycorrhizal middle European forest plants. PLoS One 10:e0124752.

Mandyam K, Jumpponen A. 2005. Seeking the elusive function of the root-colonising dark septate endophytic fungi. Stud Mycol 53:173–89.

Marhan S, Scheu S. 2006. Mixing of different mineral soil layers by endogeic earthworms affects carbon and nitrogen mineralization. Biol Fertil Soils 42:308–14.

McCormack ML, Dickie IA, Eissenstat DM, Fahey TJ, Fernandez CW, Guo D, Erik A, Iversen CM, Jackson RB, Leppalammi-Kujansuu J, Norby RJ, Phillips RP, Pregitzer KS, Pritchard SG, Rewald B, Zadworny M. 2015. Redefining fine roots improves understanding of below-ground contributions to terrestrial biosphere processes. New Phytol 207:505–18.

McGonigle TP, Miller MH, Evans DG, Fairchild GL, Swan J a. 1990. A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. New Phytol 115:495–501.

McLean M a., Migge-Kleian S, Parkinson D. 2006. Earthworm invasions of ecosystems devoid of earthworms: Effects on soil microbes. Hendrix PF, editor. Biol Invasions 8:1257–73.

McLean MA, Parkinson D. 2000. Introduction of the epigeic earthworm *Dendrobaena octaedra* changes the oribatid community and microarthropod abundances in a pine forest. Soil Biol Biochem 32:1671–81.

Murray BD, Webster CR, Bump JK. 2013. Broadening the ecological context of ungulate – ecosystem interactions : the importance of space, seasonality, and nitrogen. 94:1317–26.

Nuzzo VA, Maerz JC, Blossey B. 2009. Earthworm invasion as the driving force behind plant invasion and community change in northeastern North American forests. Conserv Biol 23:966–74.

Nuzzo V, Davalos A, Blossey B. 2017. Assessing plant community composition fails to capture impacts of white-tailed deer on native and invasive plant species. Ann Bot

9:plx026.

Olsson PA, Burleigh SH, Aarle IM Van, Olsson PA. 2002. The influence of external nitrogen on carbon allocation to *Glomus intraradices* in monoxenic arbuscular mycorrhiza. :677–86.

Paré D, Bernier B. 1989. Origin of the phosphorus deficiency observed in declining sugar maple stands in the Quebec Appalachians. Can J For Res 19:24–34.

Paudel S, Longcore T, Macdonald B, McCormick MK, Szlavecz K, Wilson GW, Loss SR. 2016a. Belowground interactions with aboveground consequences: Invasive earthworms and arbuscular mycorrhizal fungi. Ecology 97:605–14.

Paudel S, Longcore T, MacDonald B, McCormick MK, Szlavecz K, Wilson GW, Loss SR. 2016b. Belowground interactions with aboveground consequences: Invasive earthworms and arbuscular mycorrhizal fungi. Ecology 97:605–14.

Peterson AC, Hendrix PF, Haydu C, Graham RC, Quideau SA. 2001. Single-shrub influence on earthworms and soil macroarthropods in the southern California chaparral. Pedobiologia (Jena) 45:509–22.

Rabatin SC, Stinner BR. 1988. Indirect effects of interactions between VAM fungi and soil-inhabiting invertebrates on plant processes. Agric Ecosyst Environ 24:135–46.

Rearick D, Kintz L, Burke KL, Ransom TS. 2011. Effects of white-tailed deer on the native earthworm, *Eisenoides carolinensis*, in the southern Appalachian Mountains, USA. Pedobiologia (Jena) 54:S173–80.

Resner K, Yoo K, Sebestyen SD, Aufdenkampe A, Hale C, Lyttle A, Blum A. 2015. Invasive earthworms deplete key soil inorganic nutrients (Ca, Mg, K, and P) in a northern hardwood forest. Ecosystems 18:89–102.

Rooney TP, Waller DM. 2003. Direct and indirect effects of white-tailed deer in forest ecosystems. For Ecol Manage 181:165–76.

Rossow LJ, Bryant JP, Kielland K. 1997. Effects of above-ground browsing by mammals on mycorrhizal infection in an early successional taiga ecosystem. Oecologia 110:94–8.

Russell MB, Woodall CW, Potter KM, Walters BF, Domke GM, Oswalt CM. 2017. Interactions between white-tailed deer density and the composition of forest understories in the northern United States. For Ecol Manage 384:26–33.

Seki Y, Okuda K, Koganezawa M. 2014. Indirect effects of sika deer on Japanese badgers. Mammal Study 39:201–8.

Shelton AL, Henning J a., Schultz P, Clay K. 2014. Effects of abundant white-tailed deer on vegetation, animals, mycorrhizal fungi, and soils. For Ecol Manage 320:39–49.

Shipley B. 2000. A new inferential test for path models based on directed acyclic graphs. Struct Equ Model A Multidiscip J 7:206–18.

Shipley B. 2009. Confirmatory path analysis in a generalized multilevel context. 90:363–8.

Simkin SM, Allen EB, Bowman WD, Clark CM, Belnap J, Brooks ML, Cade BS, Collins SL, Geiser LH, Gilliam FS, Jovan SE, Pardo LH, Schulz BK, Stevens CJ, Suding KN, Throop HL, Waller DM. 2016. Conditional vulnerability of plant diversity to atmospheric nitrogen deposition across the United States. Proc Natl Acad Sci 113:4086.

Simmons W, Dávalos A, Blossey B. 2015. Forest successional history and earthworm legacy affect earthworm survival and performance. Pedobiologia (Jena) 58:153–64.

Smith MS, Fridley JD, Goebel M, Bauerle TL. 2014. Links between belowground and aboveground resource-related traits reveal species growth strategies that promote invasive advantages. PLoS One 9: e104189.

Smith SE, Jakobsen I, Grønlund M, Smith FA. 2011. Roles of arbuscular mycorrhizas in plant phosphorus nutrition: interactions between pathways of phosphorus uptake in arbuscular mycorrhizal roots have important implications for understanding and manipulating plant phosphorus acquisition. Plant Physiol 156:1050–7.

Sobral M, Silvius KM, Overman H, Oliveira LFB, Raab TK, Fragoso JMV. 2017. Mammal diversity influences the carbon cycle through trophic interactions in the Amazon. Nat Ecol Evol 1:1670–6.

Stoyke G, Currah RS. 1991. Endophytic fungi from the mycorrhizae of alpine ericoid plants. Can J Bot 69:347–52.

Suarez ER, Pelletier DM, Fahey TJ, Groffman PM, Bohlen PJ, Fisk MC. 2004. Effects of exotic earthworms on soil phosphorus cycling in two broadleaf temperate forests. Ecosystems 7:28–44.

Toju H, Sato H, Tanabe AS. 2014. Diversity and spatial structure of belowground plant – fungal symbiosis in a mixed subtropical forest of ectomycorrhizal and arbuscular mycorrhizal plants. PLoS One 9:e86566.

Vierheilig H, Coughlan AP, Wyss URS, Recherche C De. 1998. Ink and vinegar, a simple staining technique for arbuscular mycorrhizal fungi. Appl Environ Microbiol 64:5004–7.

Vitousek PM, Cassman KEN, Cleveland C, Field CB, Grimm NB, Hole W. 2002. Towards an ecological understanding of biological nitrogen fixation. Biogeochemistry 57:58:1–45.

Waller DM. 2014. Effects of deer on forest herb layers. In: Gilliam FS, Roberts MR, editors. The herbaceous layer in forests of eastern North America. pp 369–99.

Wang R, Wang Q, Zhao N, Xu Z, Zhu X, Jiao C, Yu G, He N. 2017. Different phylogenetic and environmental controls of first-order root morphological and nutrient traits: evidence of multidimensional root traits. Community Ecol. 32:29-39

Watmough SA, Meadows MJ. 2014. Do earthworms have a greater influence on nitrogen dynamics than atmospheric nitrogen deposition? Ecosystems 17:1257–70.

Wiegmann SM, Waller DM. 2006. Fifty years of change in northern upland forest

understories: Identity and traits of ‘winner’ and ‘loser’ plant species. Biol Conserv 129:109–23.

Wurst S, Rillig MC. 2011. Additive effects of functionally dissimilar above- and belowground organisms on a grassland plant community. 4:221–7.

Yang N, Schützenmeister K, Grubert D, Jungkunst HF, Gansert D, Scheu S, Polle A, Pena R. 2015. Impacts of earthworms on nitrogen acquisition from leaf litter by arbuscular mycorrhizal ash and ectomycorrhizal beech trees. Environ Exp Bot. 120:1-7.

Chapter 4

INVASIVE EARTHWORMS AND WHITE-TAILED DEER IMPACT NATIVE UNDERSTORY PLANT SURVIVAL, GROWTH AND REPRODUCTION

ABSTRACT

1. Following European settlement and farm abandonment in northeastern North America, white tailed deer and non-native earthworms have emerged as major structuring elements in forests. Rapid population growth of deer and range expansions of earthworms have occurred simultaneously with other stressors, limiting our ability to understand how various plant species decline and change in understory communities.
2. Using a 2 x 2 full factorial design in five hardwood forests, we transplanted 20 native understory plant species into plots with and without deer and earthworms, and measured plant growth, survival and reproduction over six years.
3. At the end of the experiment, earthworms increased survival of 13 of 20 species and decreased survival of five. Those transplants that survived grew taller (seven of 13 species) and wider (five of nine species) in earthworm invaded plots, including those species whose survival was negatively affected. Transplants growing in earthworm invaded plots were more likely to be attacked by insects. Earthworm effects (both negative and positive) became less pronounced over time.
4. Deer limited most species' growth, reproduction, and (to a lesser degree) survival. Plant traits, especially high foliar N, were related to lower survival in the presence of deer and earthworms. Although there was some evidence for patterns of sensitivity to worms and deer related to phylogenetic relatedness, we lacked the breadth of species to detect significant effects.

5. *Synthesis:* Deer and earthworm effects on survival changed over time and were independent of their effects on growth in many species. Earthworms limited survival of most species in the first year of the experiment, but eventually became filters, benefiting some species and impairing others. Indirect negative deer effects were observed in many tall, unpalatable species in addition to direct effects on palatable species. These findings support the hypothesis that deer limit growth for many species in the forest understory, regardless of their palatability. Plants are impacted through direct browse as a function of height, or due to non-consumptive effects.

INTRODUCTION

While "megadisturbances" such as extreme droughts, insect outbreaks, windstorms and more frequent and intense fires are easily recognized drivers of change, less discrete anthropogenic forces create shifts in composition and structure of plant communities that are less obvious (Waller et al. 2015). Temperate North American forests are experiencing broad changes in local community composition, with many species declining directly or indirectly as a result of human activities (McKinney and Lockwood 1999). Long-term plant community data and herbarium records have identified species that have increased or decreased over time, citing sensitivity to disturbance as a key attribute in their decline (Wiegmann and Waller 2006; Roberts and Gilliam 2014). While this designation is helpful in identifying species of highest conservation priority, it has limited scope for conservation and management strategies.

In northeastern forests, nitrogen deposition, climate change, invasive species (earthworms, insects, pathogens, plants), land-use change and expansion of native species (*Carex pensylvanica* Lam., *Dennstaedtia punctilobula* (Michx.) T. Moore, white tailed deer [*Odocoileus virginianus* Zimmerman, 1780]) have all been identified as drivers of declines in susceptible species (Rooney and Dress 1997; Hale et al. 2008; Royo and Carson 2008; Corio et al. 2009; Sanderson et al. 2012; Lovett et al. 2013; Herms and McCullough 2014; Waller 2014; Simkin et al. 2016). However, these disturbances and stressors are often co-occurring, limiting our ability to understand which are the drivers and which the passengers of change (MacDougall and Turkington 2005; Didham et al. 2005). Mesocosm experiments are helpful for establishing effects of individual and multiple stressors, but the small spatial and temporal scales limits their ability to explain impacts that develop over time, such as changes to soil aggregation (Underwood et al. 2005). To overcome some of these experimental shortcomings, we established a 2 x 2 full factorial field study using experimental plantings in

a suite of five forests, with and without exposure to white tailed deer and invasive earthworms.

With land use change and release from top-down control by predators, deer populations have grown rapidly, becoming a major structuring force in northeastern North American forests (Rooney 2001; Rooney and Waller 2003; Côté et al. 2004; Wiegmann and Waller 2006; Waller 2014; Dávalos et al. 2015a). Deer preferentially browse palatable, nutritious species of understory plants, particularly large, reproductive individuals (Anderson 1994). Consequences of deer browse depend on life history traits of plant species, including meristem location, existence of lack of alternative reproductive strategies, root storage and re-sprout capacity (Gilliam 2014; Martin et al. 2015). Non-consumptive effects of deer affecting nutrient cycling, soil compaction and mycorrhizal networks have been identified, suggesting that the idea that deer always benefit unpalatable species is incomplete (Seagle 2003; Bressette et al. 2012; Shelton et al. 2014; Sabo et al. 2017).

Northeastern North American forests have developed in the absence of earthworms since the Wisconsin glaciation (James 1995; Steinberg et al. 1997) and typically exhibit well-developed surface organic soil horizons. Earthworm-invaded forests are fundamentally changed ecosystems, largely driven by the wide-reaching consequences of earthworms consuming the forest floor and mixing it into underlying mineral soil (Bohlen et al., 2004; Hendrix et al., 2008; Eisenhauer, 2010). The combination of accelerated nutrient cycling and increased water infiltration through macropores created by earthworm borrows can cause nutrient leaching away from rooting zones (Shipitalo et al. 2004; Suárez et al. 2004; Szlavecz et al. 2006; Hale et al. 2008; Eisenhauer et al. 2012; Dobson et al. 2017a). Invasive earthworms are simultaneously acting as detritivores, herbivores, parasite hosts, prey and competitors, earthworms interact with a large number of existing and newly introduced species in their new habitats (Wurst and Jones 2003; Edwards 2004; Maerz et al. 2009;

Nuzzo et al. 2009; Ransom 2012a; Gilbert et al. 2014). Many species of plant, bird, amphibian and arthropod that rely on forest floor habitat are negatively affected by earthworms (Gundale 2002; Hale et al. 2006b; Loss et al. 2012; Loss and Blair 2014; Shelton et al. 2014). Earthworm invasion promotes species that can take advantage of altered forest floor conditions or use earthworms as a food source (Hale et al. 2008; Powers and Nagel 2008; Madritch and Lindroth 2009; Maerz et al. 2009; Nuzzo et al. 2009). However, benefits from rapidly mineralized nutrients or prey supplement are often fleeting as the organic horizon microhabitat is lost (Maerz et al. 2009; Loss et al. 2012).

We studied deer and earthworm effects on forest plants independently and together because above and belowground community dynamics are intrinsically linked (Bardgett and Wardle 2003; Kardol et al. 2014; Buchkowski and Schmitz 2015; Jing et al. 2015; Kuebbing et al. 2015). Many of our study species have evolutionary strategies to cope with browse that rely on resorption of organic matter and mineral nutrients into their root systems and extended dormancy in an intact understory microhabitat (Gilliam 2014). Disruption of these strategies could depress some species' ability to buffer against increased herbivory. Both deer and earthworms have been implicated in increasing surface soil bulk density, decreasing litter depth, limiting soil arthropod communities, and disrupting mycorrhizae, although these effects are not universal (Bressette et al. 2012; Shelton et al. 2014; Paudel et al. 2016a).

In addition to identifying individual species responses to deer and earthworms, we assessed whether closely related species or species with shared traits respond similarly. Negative impacts of deer and earthworms on families such as Liliceae have been noted (Anderson 1994; Rooney and Gross 2003; Knight 2004; Hale et al. 2006b; Frelich et al. 2006; Dávalos et al. 2014; Dobson and Blossey 2015), but to date, studies have not had the species pool standardization or breadth to explicitly test whether this pattern is broadly supported or merely driven by a few highly studies species, such as *Trillium erectum*. For this

study, we chose subclass as the taxonomic unit to optimize both the number of species within a group and number of groups. Alternatively, what is perceived as a shared taxonomic response could instead be associated with certain plant traits. The Leaf Economic Spectrum (LES) has been useful in identifying shared traits of species response to other stressors, such as drought and fire (Ackerly 2004). Traits along the LES are related to a productivity-persistence trade-off (Reich 2014), which is relevant for species experiencing above and belowground stresses that differ within and among years. Additionally, insect herbivores may use traits to recognize more nutritious food sources (Pérez-Harguindeguy et al. 2003). We included specific leaf area (SLA) data from the TRY database (http://www.try-db.org) as a representative trait of the LES because its variation is highly correlated with a broad number of other plant traits (Kattge et al. 2011; Osnas et al. 2013; Reich 2014). We included foliar N reported in TRY, because both above and belowground herbivores can recognize N-rich food sources; high N can be associated with defense compounds; and evergreen species with low N are browsed heavily in the winter (Sinclair 1975; Bryant et al. 1983; Valentine and Heck 2001; Price et al. 2011).

Surveys of decades-old deer exclosures find that deer effects persist, and propagate up trophic levels (Nuttle et al. 2011; Nuttle et al. 2014; Shelton et al. 2014). We expected similar legacy effects from earthworm invasions, as existing seedlings and seeds are desiccated with the removal of the forest floor (Hale et al. 2006b). Therefore, because seedbanks are likely depauperate of the most sensitive species, we planted seedlings instead of assessing deer and earthworm effects on existing communities. Furthermore, this allowed us to standardize the age and species composition of the individuals we were following. Data from the first growing season of this experiment showed earthworms limiting the establishment of most plant species (Dobson and Blossey 2015). However, biotic and abiotic variables can impact plants differently at different life stages. Through this experiment we sought to understand

the effectiveness of active restoration of these species under fundamentally changed forest conditions. We tested three hypotheses: (H1) Earthworms and deer have direct, interactive effects on seedling survival, growth, reproduction and probability of insect attack; (H2) Plants in the same subclass respond similarly to earthworms and deer; (H3) Plants with shared traits respond similarly to earthworms and deer; (H4) Seedling survival in the first growing season is indicative of later performance in earthworm invaded habitats, but deer will have increasingly negative impacts.

MATERIALS AND METHODS

Study area

As part of a long-term experiment on the effects of deer and earthworms we selected five forest in the Allegheny section of the Appalachian Plateau at approximately 42°N, 76°W (Bobolink Hill, Connecticut Hill Wildlife Management Area, Hammond Hill State Forest, Ringwood Preserve and Yellow Barn State Forest). All forests had closed canopies (Leaf Area Index 5-7), dominated by mature *A. saccharu*m, *Fraxinus americana* L.*, Fagus grandifolia* Ehrh. and *Quercus rubra* L. Soils were acidic (pH 3.9 – 5.0) Fragiaquepts and Dystrochrepts in the Mardin and Volusia series (SoilWeb, USDA-NRCS & UC Davis California 2010). Land use history varied from uncleared, sporadically logged forest at Ringwood preserve to cleared and actively (Connecticut Hill Wildlife Management Area, Yellow Barn State Forest) to passively (Hammond Hill State Forest, Bobolink Hill) reclaimed former farmland. Detailed environmental, soil and land use history is explored in Dobson and Blossey (2015), Dobson et al. (2017) and Richardson et al. (2018).

Treatments

To test effects of white-tailed deer and invasive earthworms on native understory plant communities we set up a 2 x 2 factorial experiment replicated at each forested site. Each site contained four 50 x 50 m plots (- Deer - Earthworm; + Deer - Earthworm; - Deer + Earthworm; + Deer + Earthworm). While we do not have deer densities for our forests, densities in adjacent counties range from 3.6 – 11.6 deer km^{-2} , but can reach 22 deer km^{-2} (Hunn 2007; Boulanger et al. 2014; Russell et al. 2017).

Deer exclusion plots were 10 – 100m from deer access plots and earthworm invaded plots were 0.5 – 2 km from uninvaded plots. Exclosures were installed in 2011 and consisted of plastic mesh fence (2.5-m-high; deerbusters.com, Standard perimeter fencing, Wayesnboro, PA) attached to two cables strung between trees. We established 20 planting grids in each plot, each with space for 20 transplants (one of each species).

We sampled earthworms in 10 0.5 m^2 quadrats along two diagonal transects within each plot in September 2011 (before fence construction) and again in 2012, 2013 and 2015. We used liquid mustard extraction (3 g powdered mustard in 3.79 L tap water), poured over sampling quadrats with leaf litter removed (Lawrence & Bowers 2002). We collected all surfacing earthworms and preserved them in formalin for 48 h before transferring them into ethanol for storage. We weighed and, wherever possible, identified each earthworm to species (Reynolds 1977; Hale 2007). We pooled all 10 samples from within each plot and dried them at 60 °C for 72 h to determine dry biomass. Earthworm-invaded plots at Connecticut Hill and Hammond Hill had exposed mineral soil, while Ringwood, Yellow Barn and Bobolink Hill retained a thin and discontinuous forest floor. The reason for and timing of earthworm invasion of these forests is unknown, distance to agricultural clearings (Suarez et al. 2006b) and forest age (Simmons et al. 2015) were the best predictors of earthworm presence and biomass in proximate forests.

Seedling establishment

We germinated seeds of 20 species of native plants from local (seed collected within 100km) genotypes to represent a breadth of life histories. Species included *Actaea rubra* (Aiton) Willd., *Agrimonia gryposepala* Wallr., *Allium tricoccum* Aiton, *Arisaema tryphyllum* (L.) Schott, *Brachyelytrum erectum* (Schreb. ex Spreng.) P. Beauv., *Carex radiata* (Wahlenb.) Small, *Caulophyllum thalictroides* (L.) Michx., *Fraxinus americana* L., *Geranium maculatum* L., *Geum canadense* Jacq., *Maianthemum racemosum* (L.) Link, *Polygonatum biflorum* (Walter) Elliott, *Polygonum virginianum* L., *Sanguinaria canadensis* L., *Thalictrum dicocum* L., *Tiarella cordifolia* L., and *Trillium erectum* L. We germinated seedlings in potting soil (BX General Purpose Pro-mix, Premier Brands Inc., Riviere-du-Loup, Quebec, Canada). We selected the largest individuals to transplant into new potting soil (BX General Purpose Pro-mix mycorrhizae, Premier Brands Inc., Riviere-du-Loup, Quebec, Canada) inoculated with *Glomus intaradices* Schenck & Sm. mycorrhizae in 6 x 3.7 x 6 cm or 6 x 6 x 5.3 cm cell packs. We grew seedlings on an elevated table outdoors under a shade tent to restrict deer and earthworm access.

We transplanted seedlings into the planting grids during spring 2012, and spring/fall 2013 (Appendix III.1). Planting grids consisted of two 10 m long transects separated by 1m, with transplants every 1m. In total, we planted 200 - 400 individuals of 21 species across five forested sites as detailed below (n=7600 seedlings; APPENDIX III.1). In 2013 we added to the experiment *Quercus rubra* L. seedlings and ferns to the experiment, including *Adiantum pedatum* L., *Dryopteris marginalis* (L.) A. Gray and *Polystichum acrostichoides* (Michx.) Schott. *Quercus* seedlings were germinated from acorns in the spring and transplanted from 3.8 cm diameter Conetainers (Stuewe and Sons, Corvallis, Oregon, USA) with the same Pro-mix potting soil. We planted all ferns bare-root (Southern Tier Consulting, West Clarksville,

NY), and with the exception of *Polystichum*, we planted only belowground tissue. When ferns emerged the following year (2014), we observed several *Dryopteris intermedia* (Muhl. Ex Willd.) A. Gray were planted in place of *Dryopteris marginalis*, and this is included as an additional variable where significant (removed where not significant) in all statistical analyses. On the day of planting we collected species-specific information about initial plant size, including height to the topmost node, plant width, leaf width and number of leaves, stems, fronds, flowers or culms.

Seedling assessment

In 2012, we returned after approximately two weeks to replace individuals that had died from transplant shock. In 2013-2017, we returned in late May and again in early August to assess survival, growth and reproduction. We considered a plant present if it was visible above the leaf litter, and retroactively scored dormant individuals as alive if they re-appeared in a later year. Growth measurements for each species were slightly different to capture the most appropriate metric. We measured height to the highest non-reproductive node in most plants and the highest point on the plant in graminoids and ferns. We measured width as the widest point across *Caulophyllum, Actaea,* and *Adiantum*, but otherwise measured the width of the largest leaf. Measurements of reproduction were species-specific, including number of flowering culms/stems in *Carex* and *Tiarella,* flowering height in *Agrimonia* and *Geum*, flower gender in *Arisaema* and number of flowers for all other flowering plants. We did not include reproductive status of ferns because many were large, mature individuals at planting. At each transplant assessment, we noted presence/absence of insect herbivory and calculated the probability of insect herbivory on surviving individuals. While other forms of insect damage (phloem feeding, flower damage) were intermittently present, only leaf damage was common enough to be analyzed statistically.

Statistical analysis

To test direct, interactive effects of deer and earthworms on seedlings (H1), we developed generalized linear mixed models (GLMM) for binomial responses (survival, likelihood of flowering and insect herbivory) and linear mixed models for Gaussian responses (width, height). Probability of reproduction was only sufficient to perform statistical analysis on *Carex*. Three additional species (*Gerum, Geranium and Polygonum*) had <30 individuals total surviving at the end of the experiment, allowing us to model survival but not growth or reproduction. To determine whether interactive effects changed over time, we began with a three-way interaction between Deer, Earthworm presence and Year. Where it was not significant, we dropped this interaction and tested second level interactions between Year, Earthworms and Deer. For the final model, we dropped nonsignificant two-way interactions with Year, but retained the Deer x Earthworm interaction, as it was the hypothesis we were explicitly testing. We included Plot and Plot within Site as random effects to reflect the hierarchical experimental design and prevent pseudoreplication, and one species-specific measurement (height, width, number of culms) to represent initial size at transplanting (Millar and Anderson 2004).

We used a Principal Component Analysis (PCA) to explore shared ancestry (H2) and traits (H3) between species responding to deer and earthworms. Specific leaf area (SLA) and % leaf N are traits that represent different growth strategies due to their coordinated variation with a broad number of other plant traits (Osnas et al. 2013; Reich 2014). Instead of testing traits of our experimental transplants (whose trait values could be impacted by our treatments), we extracted trait information from TRY and performed the PCA with a matrix of 19 of our 20 study species. We excluded *Carex,* which did not have any entries for our variables in TRY, and included data for *Dryopteris intermedia* only (not *D. marginalis*).

Where more than one entry per species was present in TRY, we calculated the mean of all entries. Each species was classified by subclass according to the United States Department of Agriculture PLANTS Database (https://plants.usda.gov); however, several taxa (Arecidae, Caryophyllidae, Commelinidae, Hamamelididae) had only one example, limiting our ability to form conclusions about them. We chose subclass as the taxonomic unit, and included survival response to deer and earthworms from our study. Survival response was calculated as the predicted response at the end of the experiment using the GLMMs described above. Next, we used AICc to test the best models from the PCA. Starting with the null model, we added parameters one at a time, choosing the models with the lowest AICc as the best models (Burnham et al. 2002). We fitted all mixed models with package lme4 (Douglas et al. 2015), generated model predictions with emmeans (Russell 2017), calculated AICc with AICcmodavg (Mazerolle 2017) and PCA with ggplot2 (Wickham 2009) in the statistical program R (R Core Team 2014).

RESULTS

Earthworms

Earthworm populations fluctuated annually, with the highest densities appearing in different plots in different years (Appendix III.2). Deer exclusion did not impact dry earthworm biomass ($t = 0.69$, $df = 1$, $P=0.49$) or densities ($t= -0.74$, $df = 1$, $P=0.46$). Invaded plots had diverse earthworm species assemblages dominated by *Lumbricus terrestris* L., *Lumbricus rubellus* Hoffmeister and *Aporrectodea* sp., Savigny, with smaller populations of *Octolasion* sp. Savigny, *Dendrobaena octaedra* Savigny and *Dendrodrillus rubidus* Savigny. *Amynthas agrestis*, Goto and Hatai appeared in 2013, but remained at low population levels (<1 individual m^{-2}) until the final year of the experiment.

Seedling survival

Four of our 20 plant species had very low survival, with < 5% of individuals remaining at the end of the experiment. Ferns such as *Dryopteris* had high survival (~70% total), including several (earthworm-invaded) plots where all individuals survived. Larger transplants of a species had higher survival in 15 of 20 species (Appendix III.3), grew taller in eight of 12 species (Appendix III.4) and wider in seven of nine species (Appendix III.5). All of the ferns, particularly *Dryopteris* and *Polystichum*, had very high survival, and were planted as large, established transplants in contrast to the small seedlings of other species. Smaller transplants were never associated with higher survival. In the open (deer-access) plots, *Adiantum, Brachyelytrum* and *Polystichum* became shorter over time and *Quercus* and *Sanguinaria* (in non-earthworm plots only) had narrower leaves over time (Figs 4-2 & 4-3; Appendix III.5 & III.6; Seedling photographs Appendix III.7). In the absence of deer, all species grew wider and taller over time. We observed the most deer browse on *Quercus*, (which has persistent woody stems visible after a browse event) ranging from 0-75% of surviving individuals per plot. We observed sporadic browse on *Agrimonia, Arisaema, Brachelytrum, Caulophyllum, Dryopteris, Polygonatum, Tiarella* and *Trillium*, however it was not sufficient to perform any statistical analyses.

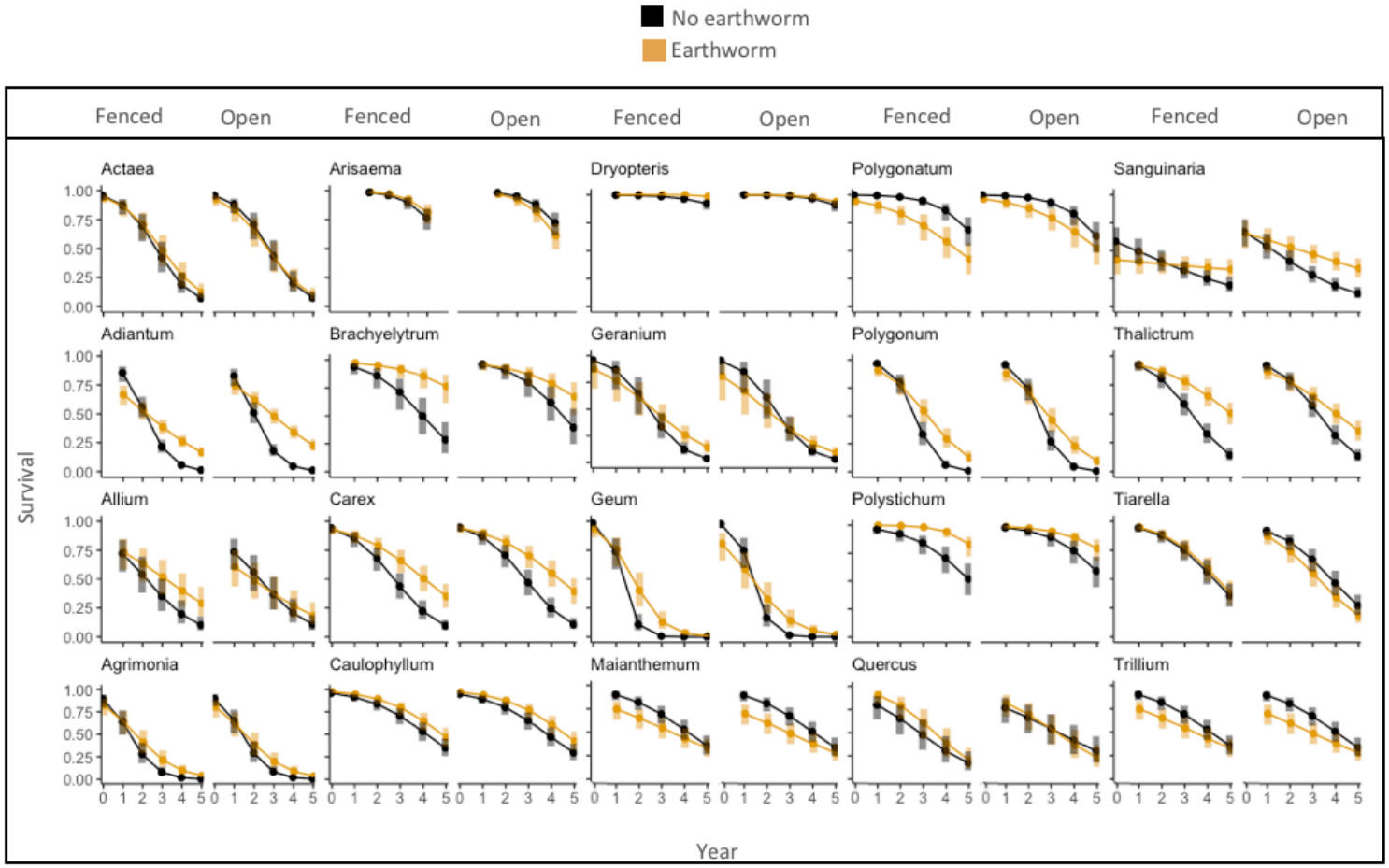
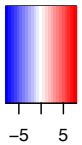


Figure 4-1 Effect of deer exclusion (left panel) vs access (right panel) and earthworm invasion (yellow) vs uninvaded (black) forest on survival of native seedling transplants (n=200–400 per species total) from 2012 – 2017 in five forests in central NY. Points and lines represent predictions from generalized linear mixed models (GLMM) with Binomial distribution and bars representing 95% confidence intervals. Site and plot within site are included as random effects. Year 0 corresponds to the predicted survival at the end of the first growing season for those seedlings planted in spring 2012 (n=8 species). Full model outputs in Appendix III.3.

Table 4-1. Parameter estimates of the effect of deer access and earthworm invasion on transplant survival (n = 200 – 400 per species) in five forested plots in central NY from 2012 - 2017. Species ordered from strongest negative effect (*Tiarella*) to strongest positive effect (*Geum*) from both deer and earthworms at the end of the experiment. Blue represents negative, red represents positive and white represents nonsignificant parameter estimates. For full models including random effects of site and plot within site, see Appendix III.3 & III.6.



Worm	WormxYear	Deer	DeerxYear	DeerxWorm	DeerxWormxYear	
0	0	0	0	−0.49	0	Tiarella
−0.35	0	0	0	0	0	Maianthemum
−1.41	0.26	0	0	0	0	Trillium
−3.16	0.42	0	0	0.63	0	Polygonatum
0	0	0	0	0	0	Arisaema
0	0	0	0	0	0	Caulophyllum
7.93	−1.23	0	0	−7.24	1.16	Dryopteris
−1.9	0.64	0	0	1.81	−0.52	Brachyelytrum
−0.66	0.29	0	−0.19	0.6	0	Sanguinaria
1.04	−0.17	0	0.23	−0.56	0	Quercus
3.74	−0.45	0	0	−3.32	0.56	Polystichum
0	0.18	0	0	0	0	Actaea
0	0.45	0	0	−0.57	0	Thalictrum
0	0.36	0	0	0	0	Carex
0	0.54	0	0	0	0	Geranium
0	0.58	0	0	−0.67	0	Allium
−2.17	1.01	0	0	0	0	Polygonum
−2.06	0.96	0	0	0.58	0	Adiantum
0	0.58	0	0	0	0	Agrimonia
−1.55	1.64	0	0.45	−0.83	0	Geum

Our treatments had markedly different effects on seedlings that changed dramatically over time. Early negative effects of earthworms (Dobson and Blossey 2015) on seedlings continued through the experiment for some species, but for other species early negative effects from earthworms disappeared or earthworm presence became beneficial over time. In the first year of the experiment, earthworm presence was nearly universally associated with lower seedling survival (Dobson and Blossey 2015). However, after several growing seasons, this trend reversed with significant positive Earthworm x Year or Earthworm x Year x Deer interactions in 13 of 20 species (Figure 4-1; Table 4-1; Appendix III.3 & III.6). At the end of the experiment, earthworms were associated with lower survival in only five species (*Maianthemum, Polygonatum, Quercus, Tiarella* and *Trillium*) compared with 13 in the first year (Figure 4-1; Appendix III.7; Dobson and Blossey 2015). The negative effects of earthworms on *Polygonatum* diminished over time (Earthworm x Year interaction; Est ± SE: 0.42 ± 0.09, Z= 4.88, P <0.001). Negative effects of worms depended on deer presence for *Tiarella* (Deer x Earthworm interaction; Est ± SE: -0.49 ± 0.21, Z= -2.38, P =0.017) and both deer and time for *Quercus* (Deer x Earthworm interaction; Est ± SE: -0.56 ± 0.22, Z= -2.60, P =0.009; Earthworm x Year interaction; Est ± SE: -0.17 ± 0.08, Z= -2.11, P =0.035). At the end of the experiment, survival of four species was highest in plots with deer access in the presence of earthworms, including *Sanguinaria* and all three fern species (Figure 4-1; Table 4-1; Appendix III.3 & III.6). Higher survival in earthworm invaded plots with deer access increased over time for the ferns *Dryopteris* and *Polystichum* (Earthworm x Deer x Year interaction; Est ± SE: *Dryopteris*, 1.16 ± 0.56, Z= 2.09, P =0.037; *Polystichum*, 0.56 ± 0.23, Z=2.43, P = 0.015). Five species (*Tiarella, Quercus, Thalictrum, Allium* and *Geum*) had a significant negative Deer x Earthworm interactions, however only *Quercus* and *Tiarella* had overall lower predicted survival with both deer and earthworms at the experiment end (Figure 4-1; Table 4-1; Appendix III.3 & III.6). Deer had no significant effects on survival of any

species through the duration of your experiment. Survival of two species (*Arisaema* and *Caulophyllum*) was not significantly affected by deer or earthworms.

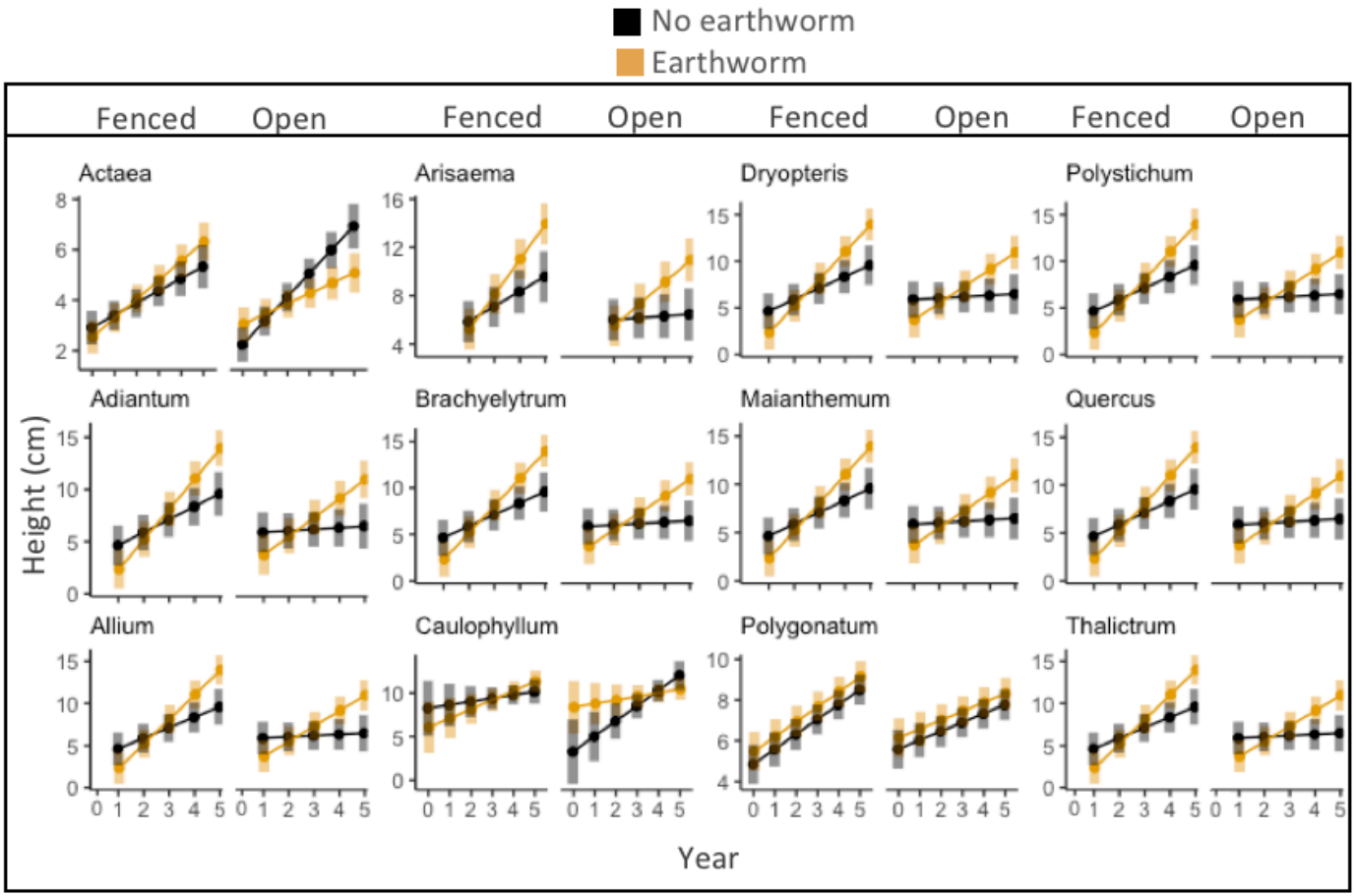
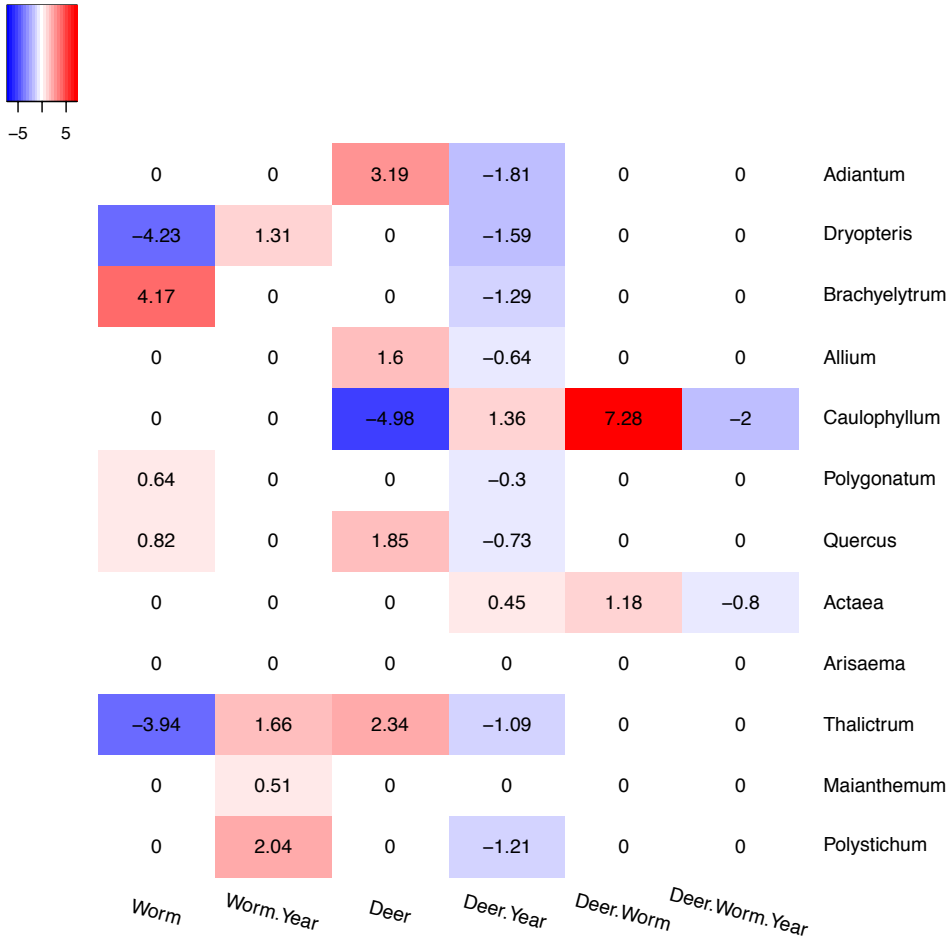


Figure 4-2. Effect of deer exclusion (left panel) vs access (right panel) and earthworm invasion (yellow) vs uninvaded (black) forest on height of native seedling transplants (n=31 - 282 per species at end of experiment) from 2012 – 2017 in five forests in central NY. Points and lines represent predictions from linear mixed models (LMM) with Gaussian distribution, and bars represent 95% confidence intervals. Site and plot within site are included as random effects. Year 0 corresponds to the predicted survival at the end of the first growing season for those seedlings planted in spring 2012 (n=8 species). Full model outputs in Appendix III.4.

Table 4-2. Parameter estimates of the effect of deer access and earthworm invasion on transplant height (n = 31 – 282 per species at end of experiment) in five forested plots in central NY from 2012 - 2017. Species ordered from strongest negative effect (*Adiantum*) to strongest positive effect (*Polystichum*) from both deer and earthworms at the end of the experiment. Blue represents negative, red represents positive and white represents nonsignificant parameter estimates. For full models including random effects of site and plot within site, see Appendix III.4 & III.6.



Seedlings Growth

While earthworms had a major effect on seedling survival, deer access was the most important predictor for growth. The height of 10 of 12 species whose height we measured was negatively affected by deer access, including two negative Deer x Year x Earthworm interactions (Fig 4-2; Table 4-2; Appendix III.4 & III.6). Apart from these three-way interactions, earthworms were associated with taller plants by the experiment in six species, either alone (*Polygonatum* and *Quercus*) or through an Earthworm x Year interaction (*Dryopteris, Thalictrum, Maiantheum* and *Polystichum*) (Fig 4-2; Table 4-2; Appendix III.4 & III.6). Deer effects on plant width were similarly strong, with four negative Deer x Year interactions and one negative Deer x Year x Earthworm interaction (Fig 4-3; Table 4-3; Appendix III.5 & III.6). Notably, *Trillium* was the only species with a significant positive Deer x Year x Earthworm interaction (Est ± SE: 0.70 ± 0.25, Z= 2.78, P =0.005). In addition to these three-way interactions, earthworm effects on width were present, but ranged from positive effects on *Quercus*; increasing over time in *Sanguinaria* and *Tiarella*; to negative effects on *Allium* (Fig 4-3; Table 4-3; Appendix III.5 & III.6). We measured *Carex* growth as number of vegetative culms, and seedlings growing with earthworms had significantly more culms over time (Earthworm x Year interaction, Est ± SE: 10.95 ± 3.00, Z= 3.65, P <0.001).

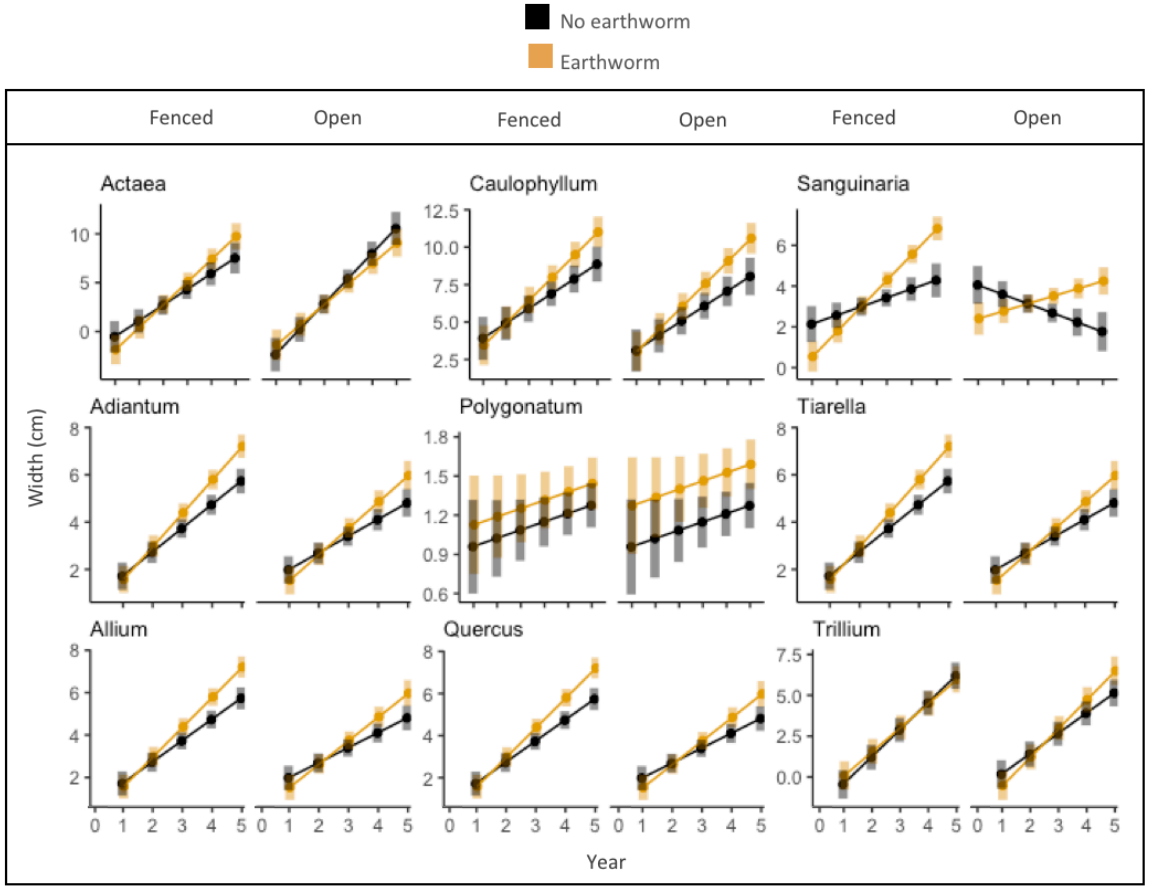
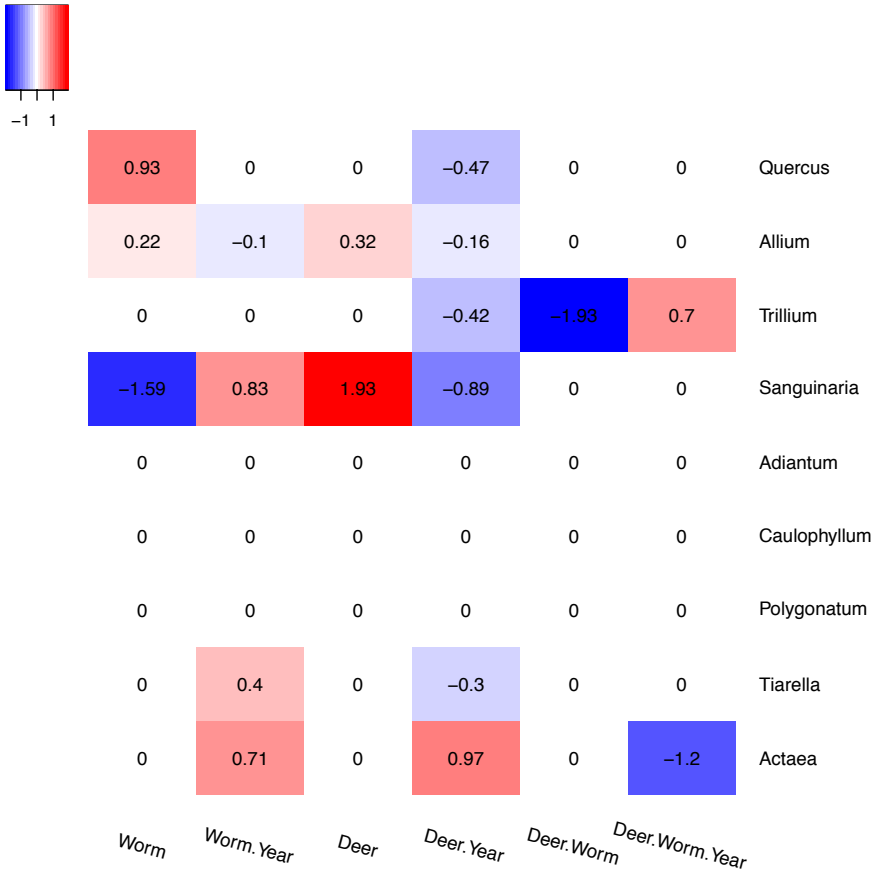


Figure 4-3. Effect of deer exclusion (left panel) vs access (right panel) and earthworm invasion (yellow) vs uninvaded (black) forest on width of native seedling transplants (n=31 - 134 per species at end of experiment) from 2012 – 2017 in five forests in central NY. Points and lines represent predictions from linear mixed models (LMM) with Gaussian distribution and bars represent 95% confidence intervals. Site and plot within site are included as random effects. Year 0 corresponds to the predicted survival at the end of the first growing season for those seedlings planted in spring 2012 (n=4 species). Full model outputs in Appendix III.5.

Table 4-3. Parameter estimates of the effect of deer access and earthworm invasion on transplant width (n = 31 – 134 per species at end of experiment) in forested plots in central NY from 2012 - 2017. Species ordered from strongest negative effect (*Quercus*) to strongest positive effect (*Actaea*) from both deer and earthworms at the end of the experiment. Blue represents negative, red represents positive and white represents nonsignificant parameter estimates. For full models including random effects of site and plot within site, see Appendix III.5 & III.6.



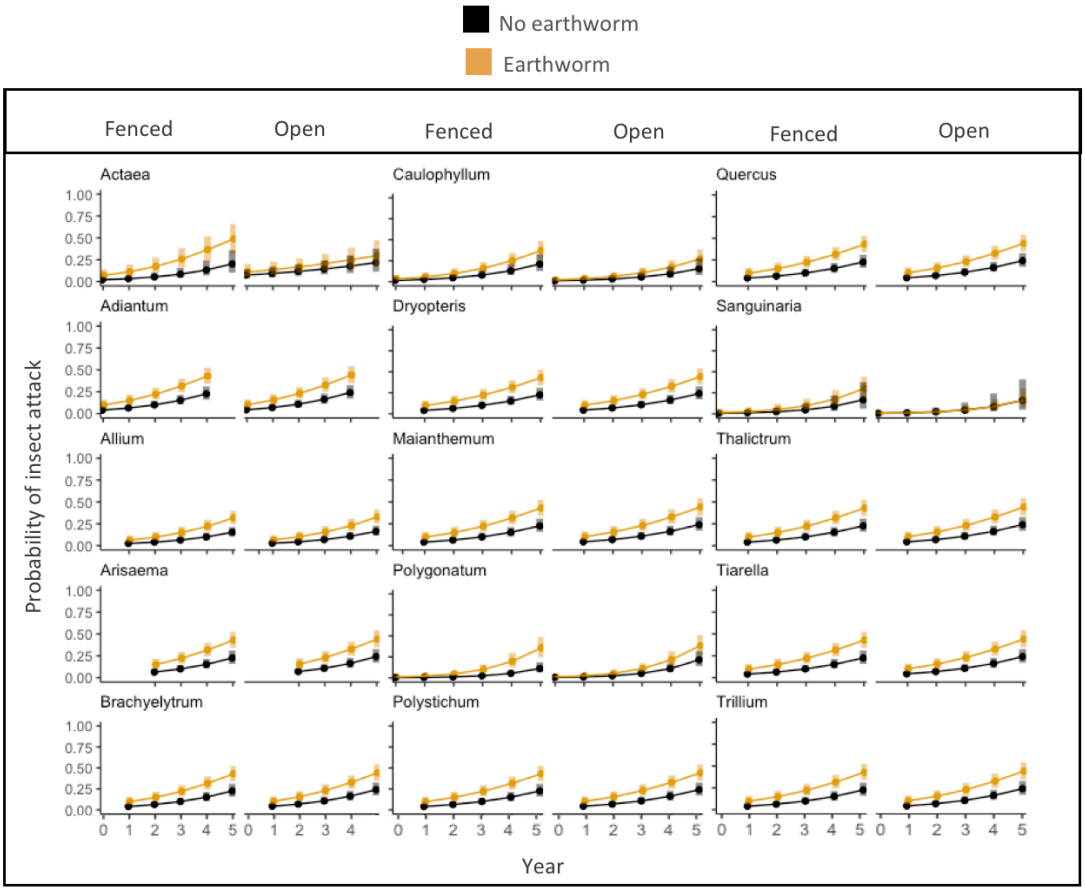


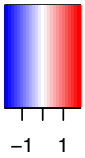
Figure 4-4 Effect of deer exclusion (left panel) vs access (right panel) and earthworm invasion (yellow) vs uninvaded (black) forest on likelihood of insect attack on native seedling transplants (n=31– 282 per species at end of experiment) from 2012 – 2017 in five forests in central NY. Predictions from generalized linear mixed models (GLMM) with Binomial distribution and bars represent 95% confidence intervals. Site and plot within site are included as random effects. Year 0 corresponds to the predicted survival at the end of the first growing season for those seedlings planted in spring 2012 (n=5 species). Full model outputs in Appendix III.9.

Flowering and Insect Attack

In addition to enhanced vegetative growth with earthworms, *Carex* were more likely to flower with earthworms (Earthworm x Year interaction, Est ± SE: 0.44 ± 0.2, Z= 2.23, P =0.026) and without deer (Deer x Year interaction, Est ± SE: -0.37 ± 0.18, Z= -2.09, P =0.037). Likelihood of flowering was not sufficient to perform statistical analysis on other species; however; it was anecdotally more likely to occur in fenced, earthworm-invaded plots (Appendix III.8). Earthworm effects on likelihood of insect attack were profound, increasing the likelihood of attack in 12 of 15 species (Fig 4-4; Table 4-4; Appendix III.6 & III.9),

although this effect was offset by a negative Deer x Earthworm interaction in *Polygonatum* and *Actaea*.

Table 4-4. Parameter estimates of the effect of deer access and earthworm invasion on likelihood of insect attack on transplants (n = 31 – 282 per species at end of experiment) in forested plots in central NY from 2012 - 2017. Species ordered from lowest risk of attack (*Polygonatum*) to elevated risk of effect (*Polystichum*) from both treatments at the end of the experiment. Blue represents negative, red represents positive and white represents nonsignificant parameter estimates. For full models including random effects of site and plot within site, see Appendix III.5 & III.6.



Worm	Worm.x.Year	Deer	Deer.x.Year	Deer.x.Worm	Deer.x.Worm.x.Year	
1.34	0	0	0	−1.43	0	Polygonatum
0	0	0	0	0	0	Allium
0	0	0	0	0	0	Brachyelytrum
0	0	0	0	0	0	Sanguinaria
1.32	0	1.37	−0.26	−0.89	0	Actaea
0.37	0	0	0	0	0	Maianthemum
0.49	0	0	0	0	0	Quercus
0.78	0	0	0	0	0	Caulophyllum
0.83	0	0	0	0	0	Dryopteris
0.97	0	0	0	0	0	Trillium
1.32	0	0	0	0	0	Arisaema
1.37	0	0	0	0	0	Thalictrum
0	0.31	0	0	0	0	Tiarella
1.83	0	0	0	0	0	Adiantum
1.17	0	0.67	0	0	0	Polystichum

Traits and Phlogeny

The first PCA axis explained 48.9% of the total variance and showed a gradient of responses to deer (PC = -0.60) and earthworms (PC = -0.59). The best model for deer effects on plant survival included % leaf N only (AICc = 40.8, R^2 = 0.18, F =3.85,df = 17, P = 0.06), where

plants with higher N were marginally negatively affected by deer (Est ± SE: -0.36 ± 0.18, t = -1.96, df = 17, P = 0.06), followed by the null model (AICc = 41.8, t = 3.3, df = 18, P =0.004). For survival response to earthworms, three models were within 2AIC (thus they cannot be considered significantly different), including N only (AICc = 82.9, R2 = 0.17, F = 3.5, df = 17, P = 0.07), the null model (AICc = 83.7, t = 3.30, df = 18, P =0.004), and SLA + N (AICc = 84.8, F = 2.35, df = 16, R2 = 0.23, P = 0.13). Although it was included in one of the top models, SLA did not significantly affect survival in the presence of earthworms (Est ± SE: -0.01 ± 0.00, t = -1.10, df = 16, P = 0.29). Species with higher % N had lower survival with earthworms in SLA + N models (Est ± SE: -1.16 ± 0.57, t = -2.05, df = 16, P = 0.05), but this was not significant in N-only models (Est ± SE: -1.05 ± 0.56, t = -1.87, df = 17, P = 0.07). As for phylogeny, although three of four Liliidae species were negatively affected by earthworms and nearly all Rosidae and Polypodidae benefited from earthworms, subclass was not included in any of the best models.

DISCUSSION

By following many species with different life histories over several growing seasons, we have developed a detailed understanding of the ways deer and earthworms affect understory plants at different life stages. Earthworms limited early establishment in most species, reducing survival of young seedlings (Dobson and Blossey 2015; Figure 4-1; Table 4-1; Appendix III.3 & III.6). This effect may be compounded by earthworm impacts on seedbanks, including granivory, burial and desiccation (Milcu et al. 2006; Drouin et al. 2014; Cassin and Kotanen 2016; Clause et al. 2016) Conversely, it may be ameliorated by improved germination conditions and increased maternal resource allocation to seeds of plants growing in the presence of earthworms (Owa et al. 2008; Nuzzo et al. 2015).

As seedlings establish, the effect of earthworms on survival and growth of understory plants changes from broadly negative to a filtering effect: benefiting some species and

impairing others. At the end of the experiment, after 4-6 years, earthworm-invaded plots had lower survival of five species (*Maianthemum, Polygonatum, Quercus, Tiarella* and *Trillium*), higher survival of 13 species (*Sanguinaria, Actaea, Brachyelytrum, Polystichum, Dryopteris, Carex, Geranium, Thalictrum, Adiantum, Allium, Agrimonia, Polygonum,* and *Geum*) and no difference for *Arisaema* and *Caulophyllum*. Negative effects of earthworms on *Quercus* survival were quantitatively small but significant (Figure 4-1). Invasive earthworms are thought to decrease seedling density of other hardwood trees which has been attributed to disruption of mycorrhizal networks and nutrient loss (Lawrence et al. 2003; Hale et al. 2006a; Bal et al. 2017; Dobson 2018).

Once a seedling becomes established over one or two growing seasons, earthworm impact changes once again. By the end of the experiment, many species were taller and wider in the presence of earthworms. Surprisingly, this included all five species whose survival was lower in earthworm plots. Further, while only two *Maianthemum* and one *Polygonatum* individuals bloomed, all were in earthworm-invaded plots. These observations suggest that earthworm impacts on survival and growth/reproduction may be decoupled. Although the importance of competition among understory plants in understory plant community dynamics is contentious (Ricard et al. 2004), it may play an indirect role through earthworm-mediated changes to soil nutrients. In an earlier investigation of these sites, we found earthworm-invaded forests with diminished organic horizons had elevated concentrations of limiting micro and mesonutrients in A horizons (Dobson et al. 2017a). Despite elevated nutrient concentrations, A horizons in earthworm-invaded soils had very few roots (Dobson et al. 2017). Taken together, this suggests earthworms create stressful rooting conditions for many native species, but others benefit from rich, relatively untapped nutrient pools in the A horizon of earthworm-invaded soils (Wilson 1988; Riegel et al. 1995; Rewald and Leuschner 2009).

Earthworms had a profound impact on plant-insect interactions. Seedlings of 12 of 15 species growing with earthworms were more likely to be attacked by insects (Fig 4-4). This pattern was apparent in the first growing season of the experiment (Dobson and Blossey 2015) and became more pronounced over time. Seedlings growing with earthworms tended to be larger, potentially attracting more herbivores (Wallace and Mansell 1976) (Figs 4-2 & 4-3). However, increased insect attack is nearly ubiquitous across plant species growing in earthworm invaded plots, suggesting additional factors are in play. Plants growing at our earthworm-invaded plots may be more attractive to insects if they contain higher concentrations of leaf Ca (as observed in Dobson et al. 2017), which is one of the most limiting nutrients to understory plants in acidic northeastern forests (Elliott 2009). Alternatively, because understory vegetation in earthworm-invaded plots was less dense (Dobson, unpublished), insects may have had limited food sources in these plots. Finally, earthworms alter physical, chemical and biological attributes of surrounding habitat and assemblages of insect herbivore larvae and their predators (Maerz et al. 2009; Ransom 2012b; Burtis et al. 2014).

Deer alone had very little impact on seedling survival, although several species had significant Deer x Earthworm or Deer x Earthworm x Year interactions. Many of our species are long-lived perennials (Appendix III.1) with the ability to re-sprout in subsequent years following browse, even when all green tissue is lost (Augustine and McNaughton 1998; Gilliam 2014). Instead of rapid seedling death, we expect deer impacts to manifest over many years as browsed seedlings return smaller and are less likely to flower (Anderson 1994; Augustine and DeCalesta 2003; Knight 2004; Waller 2014). Indeed, height, and to a lesser degree, width, were strongly negatively affect by deer access, particularly later in the experiment. *Trillium* was the only species growing wider in the presence of deer. While this may seem counterintuitive because it is a preferred food of deer (Rooney 2001; Rooney and

Gross 2003; Kraft et al. 2004; Webster et al. 2005; Knight et al. 2009), growing wider instead of taller may be an alternative re-growth strategy in *Trillium*.

Interestingly, it was not the most palatable species (Appendix III.1) that were most affected by deer, but the tallest (Fig 4-2). While several of the unaffected species (i.e. *Maianthemum* and *Polygonatum*) are palatable, very few individuals reached the 'molar zone' of 10-20cm (Waller 2014). In contrast, species such as *Brachelytrum, Dryopteris, Sanguinaria, Thalictrum* and *Tiarella* were negatively affected by deer and are relatively unpalatable species with high concentrations of secondary metabolites in both roots and shoots (Brundrett and Kendrick 1988). However, seedlings of these five species were among the largest by the end of the experiment (Figs 2 & 3). The disproportionate impact on tall species supports emerging evidence that where their abundance is sufficiently high, deer depress understory plant height and reproduction, as opposed to releasing some unpalatable species from competition (Kittredge et al. 1995; Frerker et al. 2014). Either deer are less selective (among plants in the molar zone) than previously thought, or non-consumptive effects are very important in our forests (Heckel et al. 2010), though these explanations are not mutually exclusive. Survival of species with high foliar N was most negatively affected by deer (Fig 4-5), suggesting that in forests such as ours with high deer browse pressure, deer preference is strongly tied to either size or palatability. Further, although this study was conducted over six years, it is a conservative estimate of deer effects on slow-growing perennials such as *Trillium* and *Polygonatum*, which can require a decade or more to reach the 'molar zone' and become reproductive (Cullina 2000), and declines in species abundance may lag as individuals become smaller and less likely to reproduce (Anderson 1994; Rooney and Gross 2003).

Although we expected to see synergistic impacts of deer and earthworms, this was only apparent in one of our study species (out of 20). *Tiarella* survival was unaffected by

earthworms or deer alone, but declined in the presence of both (Fig 4-1; Table 4-1). In addition to setting seed, *Tiarella* can have a prolific network of asexually spreading rhizomes. This shallow-rooted growth form is adapted to a permanent forest floor and has been identified as particularly vulnerable to earthworms, through physical disruption of the rooting network, nutrient loss to deeper soil and desiccation (Loss and Blair 2014; Drouin et al. 2016). Perhaps the additional physical disruption of root/arbuscular-mycorrhizal fungi (AMF) networks and soil compaction by deer compound these earthworm effects on the forest floor (Kardol et al. 2014; Shelton et al. 2014). *Tiarella* is not considered a preferred food of deer, and when they are browsed, meristems are protected by being low to the ground, which may be the reason we did not observe a negative effect from deer alone (Jull 2001; Waller 2014). Instead of ecological synergies from stressors, antagonism among stressors (as defined by Côté et al. (2016)) was much more common in our experiment. Several palatable species (*Geum, Allium, Thalictrum*) benefited from earthworms in the absence of deer, but had lower survival in the presence of deer. This suggests that understory plants cannot realize the benefits from earthworms in the presence of deer.

Although phylogenetic relatedness was not included in the best models of survivorship, our results indicated some mixed evidence in support of such a pattern. In particular, three of the species whose survival was negatively affected by earthworms (*Maianthemum, Polygonatum* and *Trillium*) are all in the subclass Liliidae (Figure 4-5), suggesting a phylogenetic component to earthworm impact. However, *Allium* survival was higher with earthworms (Figure 4-1), and this species is more closely related to *Polygonatum* and *Maianthemum* than is *Trillium* (Seberg et al. 2012). Similarly, three species from the subclass Rosidae (*Agrimonia, Geranium* and *Geum*) survived better with earthworms; however *Tiarella* from this subclass exhibited the opposite response. In terms of plant traits, foliar N was moderately successful at determining survival in earthworm-invaded forests,

with low foliar N species surviving better in earthworm-invaded forests (Fig 4-5). Perhaps species with higher tissue N are more prone to earthworm herbivory, as earthworms are root (and occasionally shoot) herbivores (Fisk et al. 2004a; Griffith et al. 2013; Gilbert et al. 2014; Kirchberger et al. 2015). Alternatively, foliar N could be related to another important trait that varies along the LES (Reich 2014). While this is a coarse assessment that does not differentiate between traits affecting palatability vs. resistance to browse effects, it suggests that phylogeny alone does not determine response to earthworms (H 2) and that traits are more important (H 3). Incorporating belowground traits such as root nitrogen, root architecture and presence of secondary metabolites may improve the predictive power of trait-based models specific to earthworm impacts.

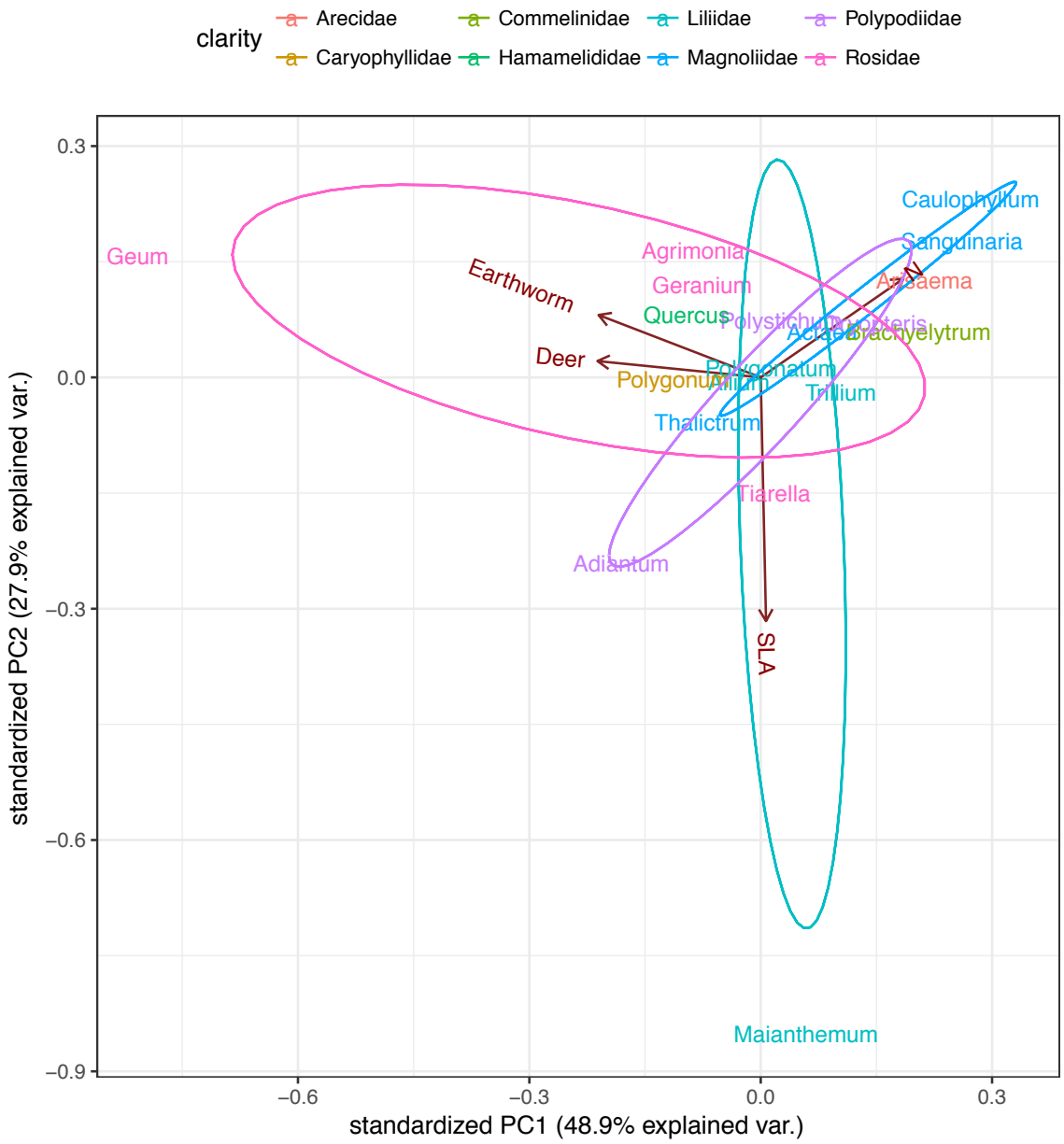


Figure 4-5 Principal Component Analysis (PCA) of the understory species subclass (color), functional traits and survival response to deer and earthworms at the end of the experiment. Trait data of 19 species collected from TRY database, subclass from USDA PLANTS database.

Our experiment focused on one generation of individuals, but we would expect deer and earthworms to have profound impacts on demography. Deer could further disrupt reproduction by eliminating networks of reproductive individuals with large, showy flowers that attract pollinators (Anderson 1994), but could benefit species that disperse through endozoochory and epizoochory. Through removal of the forest floor, earthworms depress soil arthropod populations such as ants that have a major impact on understory plant community assembly through dispersing herbaceous understory seeds (Handel et al. 1981; Kalisz et al. 1999). Indeed, in large surveys of understory plant communities in the Midwest, species whose populations have increased in the past half-century are more likely to be abiotically pollinated and dispersed (Wiegmann and Waller 2006; Frerker et al. 2014).

With the exception of shade-intolerant species (Appendix III.1) that survived poorly in all plots, many individuals of most species were able to establish in both earthworm invaded and uninvaded plots. Therefore, it is possible that after the initial wave of impacts from earthworm invasion stabilize, active restoration of the understory vegetation is possible if it is paired with deer management. Using a multi-stressor approach to conserve plant populations is the most efficient and effective course to preserve the biodiversity, resilience and ecosystem services provided by our forests.

WORKS CITED

Ackerly, D. (2004). Functional strategies of chaparral shrubs in relation to seasonal water deficit and disturbance. *Ecological Monographs*, *74*(1), 25–44.

Anderson, R. C. (1994). Height of white-flowered *Trillium* (*Trillium grandiflorum*) as an index of deer browsing intensity. *Ecological Applications*, *4*(1), 104–109.

Augustine, D. J., & DeCalesta, D. (2003). Defining deer overabundance and threats to forest communities: from individual plants to landscape structure. *Ecoscience*, *10*, 472–486.

Augustine, D. J., & McNaughton, S. J. (1998). Ungulate effects on the functional species composition of plant communities: herbivore selectivity and plant tolerance. *The Journal of Wildlife Management*, 1165–1183.

Bal, T. L., Storer, A. J., & Jurgensen, M. F. (2017). Evidence of damage from exotic invasive earthworm activity was highly correlated to sugar maple dieback in the Upper Great Lakes region. *Biological Invasions*.

Bardgett, R. D., & Wardle, D. A. (2003). Herbivore-mediated linkages between aboveground and belowground communities. *Ecology*, *84*(9), 2258–2268.

Bressette, J. W., Beck, H., & Beauchamp, V. B. (2012). Beyond the browse line: complex cascade effects mediated by white-tailed deer. *Oikos*, *121*(11), 1749–1760.

Brundrett, M. C., & Kendrick, B. (1988). The mycorrhizal status, root anatomy, and phenology of plants in a sugar maple forest. *Canadian Journal of Botany*, *66*(6), 1153–1173.

Bryant, J. P. ., III, F. . S. C., & Klein, D. R. . (1983). Carbon/nutrient balance of boreal plants in relation to vertebrate verbivory. *Oikos*, *40*(3), 357–368.

Buchkowski, R. W., & Schmitz, O. J. (2015). Detritivores ameliorate the enhancing effect of plant-based trophic cascades on nitrogen cycling in an old-field system. *Biology Letters*, *11*(4), 20141048–20141048.

Burnham, K. P., Anderson, D. R., & Burnham, K. P. (2002). *Model selection and multimodel inference: a practical information-theoretic approach* (Vol. 2). New York: Springer.

Burtis, J. C., Fahey, T. J., & Yavitt, J. B. (2014). Impact of invasive earthworms on *Ixodes scapularis* and other litter-dwelling arthropods in hardwood forests, central New York state, USA. *Applied Soil Ecology*, *84*, 148–157.

Cassin, C. M., & Kotanen, P. M. (2016). Invasive earthworms as seed predators of temperate forest plants. *Biological Invasions*, *18*(6), 1567–1580.

Clause, J., Forey, E., Eisenhauer, N., Seal, C. E., Soudey, A., Colville, L., & Barot, S. (2016). Seed selection by earthworms: chemical seed properties matter more than morphological traits. *Plant and Soil*, *413*(1–2), 97–110.

Corio, K., Wolf, A., Draney, M., & Fewless, G. (2009). Exotic earthworms of Greak Lakes forests: A search for indicator plant species in maple forests. *Forest Ecology and Management*, *258*(7), 1059–1066.

Côté, S. D., Rooney, T. P., Tremblay, J.-P., Dussault, C., & Waller, D. M. (2004). Ecological impacts of deer overabundance. *Annual Review of Ecology, Evolution & Systematics*, *35*(1), 113–147.

Cullina, W. (2000). *Wildflowers: a guide to propagating native flowers of North America*. New York: Houghton Mifflin Company.

Dávalos, A., Nuzzo, V., & Blossey, B. (2015). Forest Ecology and Management Single and interactive effects of deer and earthworms on non-native plants. *Forest Ecology and Management*, *351*, 28–35.

Dávalos, A., Simpson, E., Nuzzo, V., & Blossey, B. (2015). Non-consumptive effects of native deer on introduced earthworm abundance. *Ecosystems*, *18*(6), 1029–1042.

Didham, R. K., Tylianakis, J. M., Hutchison, M. A., Ewers, R. M., & Gemmell, N. J. (2005). Are invasive species the drivers of ecological change? *Trends in Ecology & Evolution*,

20(9), 470–474.

Dobson, A., & Blossey, B. (2015). Earthworm invasion, white-tailed deer and seedling establishment in deciduous forests of north-eastern North America. *Journal of Ecology*, *103*, 153–164.

Dobson, A. M., Blossey, B., & Richardson, J. B. (2017). Invasive earthworms change nutrient availability and uptake by forest understory plants.

Douglas, B., Maechler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, *67*(1), 1–48.

Drouin, M., Bradley, R., & Lapointe, L. (2016). Forest ecology and management linkage between exotic earthworms , understory vegetation and soil properties in sugar maple forests. *Forest Ecology and Management*, *364*, 113–121.

Drouin, M., Bradley, R., Lapointe, L., & Whalen, J. (2014). Non-native anecic earthworms (*Lumbricus terrestris* L.) reduce seed germination and seedling survival of temperate and boreal trees species. *Applied Soil Ecology*, *75*, 145–149.

Edwards, C. A. (2004). *Earthworm ecology*. (C. A. Edwards, Ed.) (2nd ed., Vol. 2). Boca Raton, Florida, USA: CRC Press.

Eisenhauer, N., Fisichelli, N. A., Frelich, L. E., & Reich, P. B. (2012). Interactive effects of global warming and "global worming" on the initial establishment of native and exotic herbaceous plant species. *Oikos*, *121*(7), 1121–1133.

Elliott, H. L. (2009). *Evaluating the influences of soil calcium and aluminum availability on ecosystem processes in northern hardwood forest*. University of Vermont.

Fisk, M. C., Fahey, T. J., Groffman, P. M., & Bohlen, P. J. (2004). Earthworm invasion, fine-root distributions, and soil respiration in north temperate forests. *Ecosystems*, *7*(1), 55–62.

Frerker, K., Sabo, A., & Waller, D. (2014). Long-term regional shifts in plant community

composition are largely explained by local deer impact experiments. *PLoS ONE*, *9*(12), 1–17.

Gilbert, K. J., Fahey, T. J., Maerz, J. C., Sherman, R. E., Bohlen, P., Dombroskie, J. J., … Yavitt, J. B. (2014). Exploring carbon flow through the root channel in a temperate forest soil food web. *Soil Biology and Biochemistry*, *76*(0), 45–52.

Gilliam, F. S. (2014). *The herbaceous layer in forests of eastern North America*. (F. S. Gilliam, Ed.) (2nd ed.). New York, New York, USA: Oxford University Press.

Griffith, B., Türke, M., Weisser, W. W., & Eisenhauer, N. (2013). Herbivore behavior in the anecic earthworm species *Lumbricus terrestris* L.? *European Journal of Soil Biology*, *55*, 62–65.

Gundale, M. J. (2002). Influence of exotic earthworms on the soil organic horizon and the rare fern *Botrychium mormo*. *Conservation Biology*, *16*(6), 1555–1561.

Hale, C. M., Frelich, L. E., & Reich, P. B. (2006). Changes in cold-temperate hardwood forest understory plant communities in response to invasion by European earthworms. *Ecology*, *87*(7), 1637–1649.

Hale, C. M., Frelich, L. E., & Reich, P. B. (2006). Changes in hardwood forest understory plant communities in response to European earthworm invasions. *Ecology*, *87*(7), 1637–1649.

Hale, C. M., Frelich, L. E., Reich, P. B., & Pastor, J. (2008). Exotic earthworm effects on hardwood forest floor, nutrient availability and native plants: A mesocosm study. *Oecologia*, *155*(3), 509–518.

Heckel, C. D., Bourg, N. A., McShea, W. J., & Kalisz, S. (2010). Nonconsumptive effects of a generalist ungulate herbivore drive decline of unpalatable forest herbs. *Ecology*, *91*(2), 319–326.

Herms, D. A., & McCullough, D. G. (2014). Emerald ash borer invasion of North America:

history, biology, ecology, impacts, and management. *Annual Review of Entomology*, *59*, 13–30.

Hooker, T. D., & Compton, J. E. (2003). Forest ecosystem carbon and nitrogen accumulation during the first century after agricultural abandonment. *Ecological Applications*, *13*(2), 299–313.

Hunn JR. 2007. Retention of logging debris to reduce deer browsing and promote forest regeneration. Senior Thesis, Department of Natural Resources, Cornell University, Ithaca NY.

James, S. W. (1995). Systematics, biogeography, and ecology of nearctic earthworms from eastern, central, southern and southwestern United States. In P. F. Hendrix (Ed.), *Earthworm ecology and biogeography in North America*. (pp. 29–52). Lewis.

Jing, X., Sanders, N. J., Shi, Y., Chu, H., Classen, A. T., Zhao, K., … He, J.-S. (2015). The links between ecosystem multifunctionality and above- and belowground biodiversity are mediated. *Nature Communications*, *6*(8159), 1–8.

Jull, L. G. (2001). Plants not favored by deer. Madison, WI: Cooperative Extension Publishing.

Kalisz, S., Hanzawa, F. M., Tonsor, S. J., Thiede, D. A., & Voigt, S. (1999). Ant-mediated seed dispersal alters spatial pattern of seed relatedness in an outcrossing population of Trillium grandiflorum. *Ecology*, *In press*(8), 2620–2634.

Kardol, P., Dickie, I. A., St. John, M. G., Husheer, S. W., Bonner, K. I., Bellingham, P. J., & Wardle, D. A. (2014). Soil-mediated effects of invasive ungulates on native tree seedlings. *Journal of Ecology*, *102*(3), 622–631.

Kirchberger, J., Eisenhauer, N., Weisser, W. W., & Türke, M. (2015). Experimental evaluation of herbivory on live plant seedlings by the earthworm *Lumbricus terrestris* in the presence and absence of soil surface litter. *Plos One*, *10*(4), e0123465.

Kittredge, D. B., Ashton, P., & Mark, S. (1995). Impact of deer browsing on regeneration in mixed stands in southern New England. *Northern Journal of Applied Forestry*, *12*(3), 115–120.

Knight, T. M. (2004). The effects of herbivory and pollen limitation on a declining population of *Trillium grandiflorum*. *Ecological Applications*, *14*(3), 915–928. doi:10.1890/03-5048

Kuebbing, S. E., Classen, A. T., Sanders, N. J., & Simberloff, D. (2015). Above- and below-ground effects of plant diversity depend on species origin: An experimental test with multiple invaders. *New Phytologist*, *208*(3), 727–735.

Lawrence, B., Fisk, M. C., Fahey, T. J., & Suarez, E. R. (2003). Influence of nonnative earthworms on mycorrhizal colonization of sugar maple (*Acer saccharum*). *New Phytologist*, *157*(1), 145–153.

Loss, S. R., & Blair, R. B. (2014). Earthworm invasions and the decline of clubmosses (*Lycopodium* spp .) that enhance nest survival rates of a ground-nesting songbird. *Forest Ecology and Management*, *324*, 64–71.

Loss, S. R., Niemi, G. J., & Blair, R. B. (2012). Invasions of non-native earthworms related to population declines of ground-nesting songbirds across a regional extent in northern hardwood forests of North America. *Landscape Ecology*, *27*(5), 683–696.

Lovett, G. M., Arthur, M. A., Weathers, K. C., & Griffin, J. M. (2013). Effects of introduced insects and diseases on forest ecosystems in the Catskill Mountains of New York. *Annals of the New York Academy of Sciences*, *1298*, 66–77.

MacDougall, A. S., & Turkington, R. (2005). Are invasive species the drivers or passengers of change in degraded ecosystems? *Ecology*, *86*(1), 42–55.

Madritch, M. D., & Lindroth, R. L. (2009). Removal of invasive shrubs reduces exotic earthworm populations. *Biological Invasions*, *11*(3), 663–671.

Maerz, J. C., Nuzzo, V. A., & Blossey, B. (2009). Declines in woodland salamander abundance associated with non-native earthworm and plant invasions. *Conservation Biology*, *23*(4), 975–981.

Martin, L. J., Agrawal, A. A., & Kraft, C. E. (2015). Historically browsed jewelweed populations exhibit greater tolerance to deer herbivory than historically protected populations. *Journal of Ecology*, *103*(1), 243–249.

Mazerolle, M. J. (2017). AICcmodavg: Model selection and multimodel inference based on (Q)AIC(c).

McKinney, M. L., & Lockwood, J. L. (1999). Biotic homogenization: A few winners replacing many losers in the next mass extinction. *Trends in Ecology and Evolution*, *14*(11), 450–453. =

Milcu, A., Schumacher, J., & Scheu, S. (2006). Earthworms (*Lumbricus terrestris*) affect plant seedling recruitment and microhabitat heterogeneity. *Functional Ecology*, *20*, 261–268.

Millar, R. B., & Anderson, M. J. (2004). Remedies for pseudoreplication. *Fisheries Research*, *70*(2–3 SPEC. ISS.), 397–407.

Nuttle, T., Ristau, T. E., & Royo, A. A. (2014). Long-term biological legacies of herbivore density in a landscape-scale experiment: forest understoreys reflect past deer density treatments for at least 20 years. *Journal of Ecology*, *102*, 221–228.

Nuttle, T., Yerger, E. H., Stoleson, S. H., & Ristau, T. E. (2011). Legacy of top-down herbivore pressure ricochets back up multiple trophic levels in forest canopies over 30 years. *Ecosphere*, *2*(1), art4.

Nuzzo, V. A., Maerz, J. C., & Blossey, B. (2009). Earthworm invasion as the driving force behind plant invasion and community change in northeastern North American forests. *Conservation Biology*, *23*(4), 966–974.

Nuzzo, V., & Andrea, D. (2015). Invasive earthworms shape forest seed bank composition. *Diversity and Distributions*, *21*, 560–570.

Osnas, J. L. D., Lichstein, J. W., Reich, P. B., & Pacala, S. W. (2013). Global leaf trait relationships: mass, area, and the leaf economics spectrum. *Science*, *340*, 741–744.

Owa, S., Moreyibi, O., Dedeke, G., Morafa S., Senjobi, B., Odunbaku, O., & Aladesida, A. (2008). Effect of over-seasoned earthworm products on seed germination: implication for early rain cropping. *Journal of Applied Sciences Research*, *4*(6), 683–687.

Pan, Y., Birdsey, R. A., Fang, J., Houghton, R., Kauppi, P. E., Kurz, W. A., … Hayes, D. (2011). A large and persistent carbon sink in the world's forests. *Science*, *333*, 988–994.

Paudel, S., Longcore, T., Macdonald, B., McCormick, M. K., Szlavecz, K., Wilson, G. W., & Loss, S. R. (2016). Belowground interactions with aboveground consequences: Invasive earthworms and arbuscular mycorrhizal fungi. *Ecology*, *97*(3), 605–614.

Pérez-Harguindeguy, N., Díaz, S., Vendramini, F., Cornelissen, J. H. C., Gurvich, D. E., & Cabido, M. (2003). Leaf traits and herbivore selection in the field and in cafeteria experiments. *Austral Ecology*, *28*(6), 642–650.

Powers, M. D., & Nagel, L. M. (2008). Disturbance dynamics influence Carex pensylvanica abundance in a northern hardwood forest. *The Journal of the Torrey Botanical Society*, *135*(3), 317–327.

Price, P. W., Denno, R. F., Eubanks, M. D., Finke, D. L., & Kaplan, I. (2011). *Insect ecology*. *Vasa*. New York: Cambridge University Press.

R Core Team. (2014). R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing.

Ransom, T. S. (2012a). Behavioral responses of a native salamander to native and invasive earthworms. *Biological Invasions*, *14*(12), 2601–2616.

Ransom, T. S. (2012b). Comparison of direct, indirect, and ecosystem engineering effects of

an earthworm on the red-backed salamander. *Ecology*, *93*(10), 2198–2207.

Reich, P. B. (2014). The world-wide " fast – slow " plant economics spectrum : a traits manifesto, 275–301.

Rewald, B., & Leuschner, Æ. C. (2009). Belowground competition in a broad-leaved temperate mixed forest : pattern analysis and experiments in a four-species stand, 387–398.

Ricard, J., Messier, C., Delagrange, S., & Beaudet, M. (2004). Do understory sapling respond to both light and below-ground competition?: a field experiment in a north-eastern American hardwood forest and a literature review. *Annals of Forest Science*, *60*(2003), 749–756.

Riegel, G. M., Miller, R. F., & Krueger, W. C. (1995). The effects of aboveground and belowground competition on understory species composition in a Pinus ponderosa forest. *Forest Science*, *41*(4), 864–889.

Roberts, M., & Gilliam, F. S. (2014). Response of the herbaceous layer to disturbance in eastern forests. In F. S. Gilliam (Ed.), *The herbaceous layer in forests of eastern North America* (2nd ed., pp. 321–339). New York: Oxford University Press.

Rooney, T., & Gross, K. (2003). A demographic study of deer browsing impacts on Trillium grandiflorum. *Plant Ecology*, *168*(2), 267–277. doi:10.1023/A:1024486606698

Rooney, T. P. (2001). Deer impacts on forest ecosystems: a North American perspective. *Forestry*, *74*(3), 201–208.

Rooney, T. P., & Dress, W. J. (1997). Species loss over sixty-six years in the ground-layer vegetation of Heart's Content, an old-growth forest in Pennsylvania USA. *Natural Areas Journal*, *17*(4), 297–305.

Rooney, T. P., & Waller, D. M. (2003). Direct and indirect effects of white-tailed deer in forest ecosystems. *Forest Dynamics and Ungulate Herbivory : From Leaf to Landscape*,

181(1–2), 165–176.

Royo, A. A., & Carson, W. P. (2008). Direct and indirect effects of a dense understory on tree seedling recruitment in temperate forests: habitat-mediated predation versus competition. *Canadian Journal of Forest Research*, *38*(6), 1634–1645.

Russell, L. (2017). emmeans: estimated marginal means, aka least-squares means.

Russell MB, Woodall CW, Potter KM, Walters BF, Domke GM, Oswalt CM. 2017. Interactions between white-tailed deer density and the composition of forest understories in the northern United States. *For Ecol Manage* 384:26–33.

Sabo, A. E., Frerker, K. L., Waller, D. M., & Kruger, E. L. (2017). Deer-mediated changes in environment compound the direct impacts of herbivory on understorey plant communities. *Journal of Ecology*.

Sanderson, L. A., McLaughlin, J. A., & Antunes, P. M. (2012). The last great forest: a review of the status of invasive species in the North American boreal forest. *Forestry*, *85*(3), 329–339.

Seagle, S. W. (2003). Can ungulates foraging in a multiple-use landscape alter forest nitrogen budgets? *Oikos*, *103*(1), 230–234.

Seberg, O., Petersen, G., Davis, J. I., Pires, J. C., Stevenson, Dennis, W., Chase, M. W., … Yohan, P. (2012). Phylogeny of the Asparagales based on three plastid and two mitochondrial genes. *American Journal of Botany*, *99*(5), 875–889.

Shelton, A. L., Henning, J. a., Schultz, P., & Clay, K. (2014). Effects of abundant white-tailed deer on vegetation, animals, mycorrhizal fungi, and soils. *Forest Ecology and Management*, *320*, 39–49.

Shipitalo, M. J., Nuutinen, V., & Butt, K. R. (2004). Interaction of earthworm burrows and cracks in a clayey, subsurface-drained, soil. *Applied Soil Ecology*, *26*(3), 209–217.

Simkin, S. M., Allen, E. B., Bowman, W. D., Clark, C. M., Belnap, J., Brooks, M. L., …

Waller, D. M. (2016). Conditional vulnerability of plant diversity to atmospheric nitrogen deposition across the United States. *Proceedings of the National Academy of Sciences*, *113*(15), 4086.

Sinclair, A. R. E. (1975). The resource limitation of trophic levels in tropical grassland ecosystems. *Journal of Animal Ecology*, *44*(2), 497–520.

Steinberg, D. A., Pouyat, R. V, Parmelee, R. W., & Groffman, P. M. (1997). Earthworm abundance and nitrogen mineralization rates along an urban-rural land use gradient. *Soil Biology & Biochemistry*, *29*(3–4), 427–430.

Suárez, E. R., Pelletier, D. M., Fahey, T. J., Groffman, P. M., Bohlen, P. J., & Fisk, M. C. (2004). Effects of exotic earthworms on soil phosphorus cycling in two broadleaf temperate forests. *Ecosystems*, *7*(1), 28–44.

Szlavecz, K., Placella, S. A., Pouyat, R. V, Groffman, P. M., Csuzdi, C., & Yesilonis, I. (2006). Invasive earthworm species and nitrogen cycling in remnant forest patches. *Applied Soil Ecology*, *32*(1), 54–62.

Underwood, N., Hambäck, P., & Inouye, B. D. (2005). Large-scale questions and small-scale data: Empirical and theoretical methods for scaling up in ecology. *Oecologia*, *145*(2), 177–178.

Valentine, J. F., & Heck, K. L. (2001). The role of leaf nitrogen content in determining turtlegrass (Thalassia testudinum) grazing by a generalized herbivore in the northeastern Gulf of Mexico. *Journal of Experimental Marine Biology and Ecology*, *258*(1), 65–86.

Waller, D. M. (2014). Effects of deer on forest herb layers. In F. S. Gilliam & M. R. Roberts (Eds.), *The herbaceous layer in forests of eastern North America* (pp. 369–399).

Waller, D. M., Ash, J., Paulson, A., & Sonnier, G. (2015). Tempering threats to temperate forests. *Science*, *350*(6262), 748.

Wickham, H. (2009). *ggplot2: Elegant Graphics for Data Analysis*. New York: Springer-

Verleg.

Wiegmann, S. M., & Waller, D. M. (2006). Fifty years of change in northern upland forest understories: Identity and traits of "winner" and "loser" plant species. *Biological Conservation*, *129*(1), 109–123. doi:http://dx.doi.org/10.1016/j.biocon.2005.10.027

Wilson, B. Y. J. B. (1988). Shoot Competition and Root Competition. *Journal of Applied Ecology*, *25*(1), 279–296.

Wurst, S., & Jones, T. H. (2003). Indirect effects of earthworms (*Aporrectodea caliginosa*) on an above-ground tritrophic interaction. *Pedobiologia*, *47*(1), 91–97.

Chapter 5
CONCLUSIONS

The goal of the research presented here was to investigate individual and combined effects of deer on understory plant communities. By implementing a variety of transplant experiments I investigated several mechanistic hypotheses for how these impacts influence plant survival, growth and reproduction in five forests in central New York State. In Chapter 2, I found earthworms cause large changes to cycles of macro, meso and micronutrients. A plants' ability to capitalize on higher concentrations of soil nutrients (such as Ca) and buffer itself against lower nutrient concentrations (such as P) in earthworm invaded plots was characteristic of a successful species. In Chapter 2, I show that in the absence of earthworms, root mass in the soil is concentrated in the A horizon, but disappears from the A horizon in earthworm-invaded forests. Species that cannot grow in stressful rooting conditions created by earthworms, but have the plasticity or adaptive capacity to grow deeper may persist in these altered forest soils. However, without intervention to restore declining or lost populations of native plants, the most successful plants in earthworm-invaded conditions are likely to be non-native species that have evolved to root directly into exposed mineral soil. These species may be able to access rich nutrient pools in the A horizon that native plant roots cannot access due their rooting morphology.

Although four herbaceous species and one tree species had lower survival in earthworm invaded plots, Chapter 4 demonstrated that many individuals were able to establish. What remains to be seen is whether, for example in *Polygonatum biflorum*, ~40% survival of transplants in earthworm-invaded plots instead of ~60% is biologically meaningful for restoration purposes. Even species such as *Polygonatum* that survived more poorly in earthworm-invaded plots grew taller, wider and were (anecdotally) more likely to reproduce. This could suggest that these transplants are able to root in the A-horizon and capitalize on high concentrations of Ca, Mg, K and S.

Taken together, these studies provide a hopeful way forward to restoring understory communities. Initial earthworm invasions rapidly change forest floor conditions, wiping out a generation of established native plants. Although earthworm control is not currently viable, we show that most shade-tolerant species can establish once forest floor conditions have stabilized. With earthworms as a vector, AMF may quickly re-colonize (Gange 1993) and the position of the forest floor may stabilize. Even when conditions stabilized, a century of intense deer browse on large, reproductive individuals leaves a depauperate seedbank, limiting natural rescue of declining native plant populations (Frelich et al. 2006). In light of this research, I propose that conservation of understory plant communities is possible through active restoration with transplants. While survival of most species was initially lower in earthworm-invaded plots, the transplants that did establish grew larger than those in intact forests. This could mean that active restoration may have the added benefit of establishing native transplants in lieu of invaders that could otherwise benefit from nutrient-rich A horizon pools.

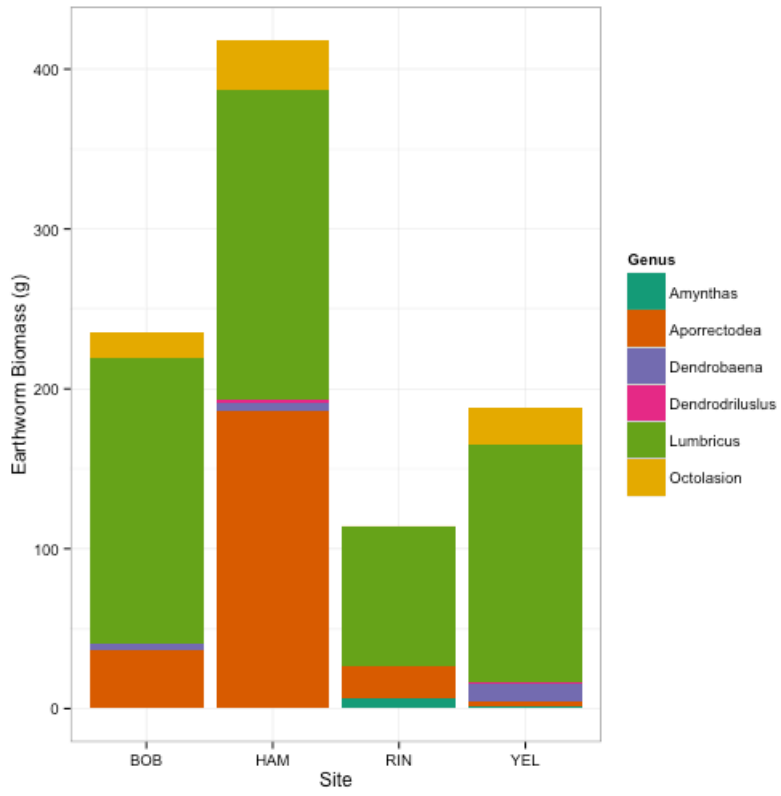
As expected in Chapter 4, species with high foliar N were most negatively affected by deer, but many species considered unpalatable were strongly negatively affected. This could be a result of non-consumptive effects of deer, such as changes to soil nutrient dynamics demonstrated in Chapter 3. Since it is mainly the tallest species that are negatively affected by deer, their diet may include unpalatable species in ecosystems where deer populations are sufficiently high to have depleted most green vegetation in the 'molar zone.' Therefore, we propose that successful restoration is possible, but must include deer management. Without deer management, seedlings will remain small, and may ultimately disappear. An active, multi-stressor approach to restoration is the best conservation strategy to maintain the biodiversity and resilience of our forests.

WORKS CITED

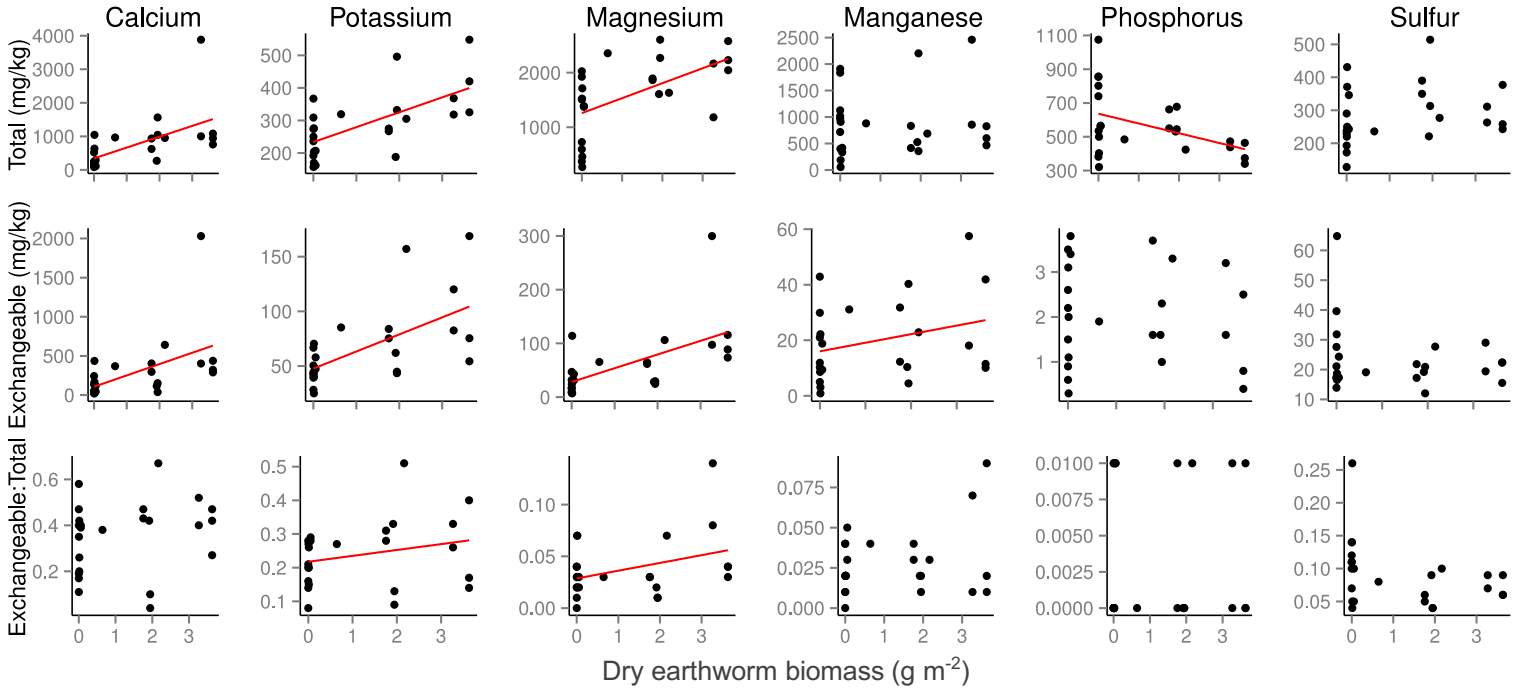
Frelich LE, Hale CM, Scheu S, Holdsworth AR, Heneghan L, Bohlen PJ, Reich PB. 2006. Earthworm invasion into previously earthworm-free temperate and boreal forests. Biol Invasions 8:1235–45.

Gange AC. 1993. Translocation of mycorrhizal fungi by earthworms during early succession. Soil Biol Biochem 25:1021–6.

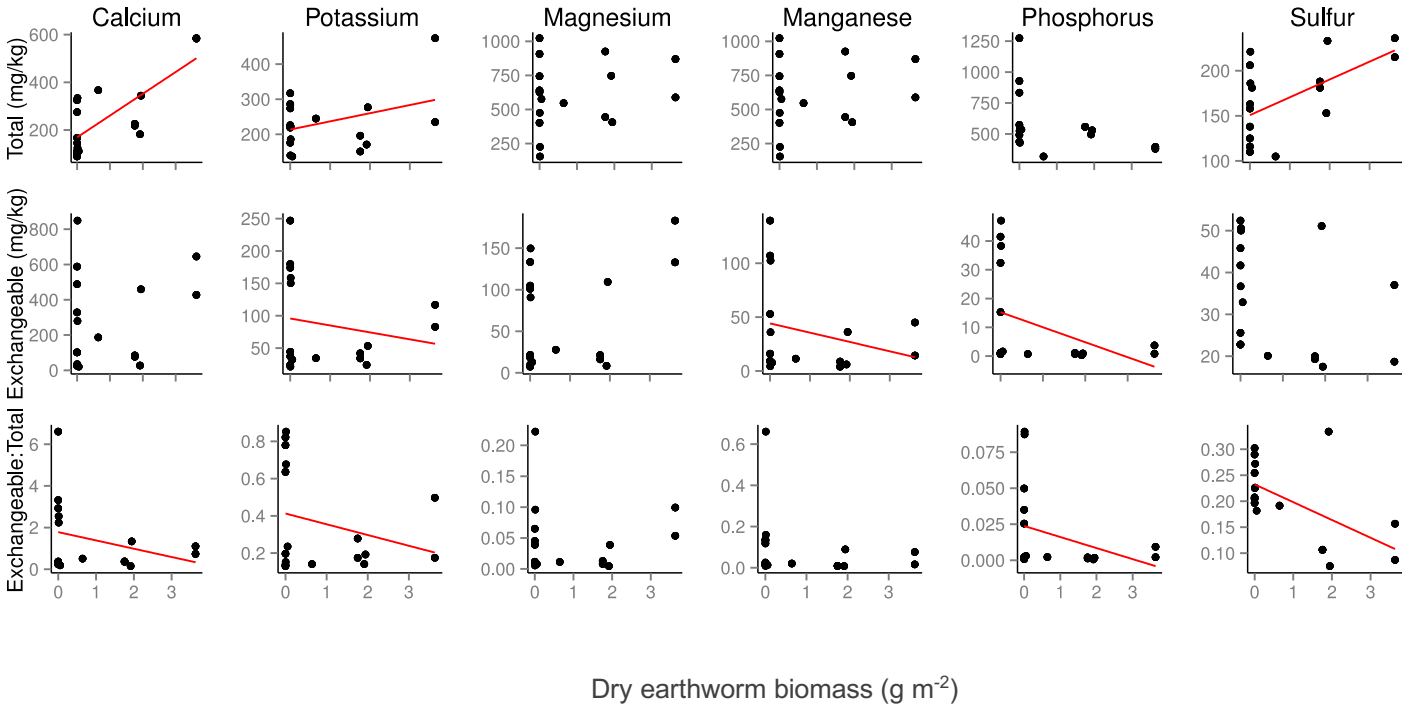
APPENDIX I



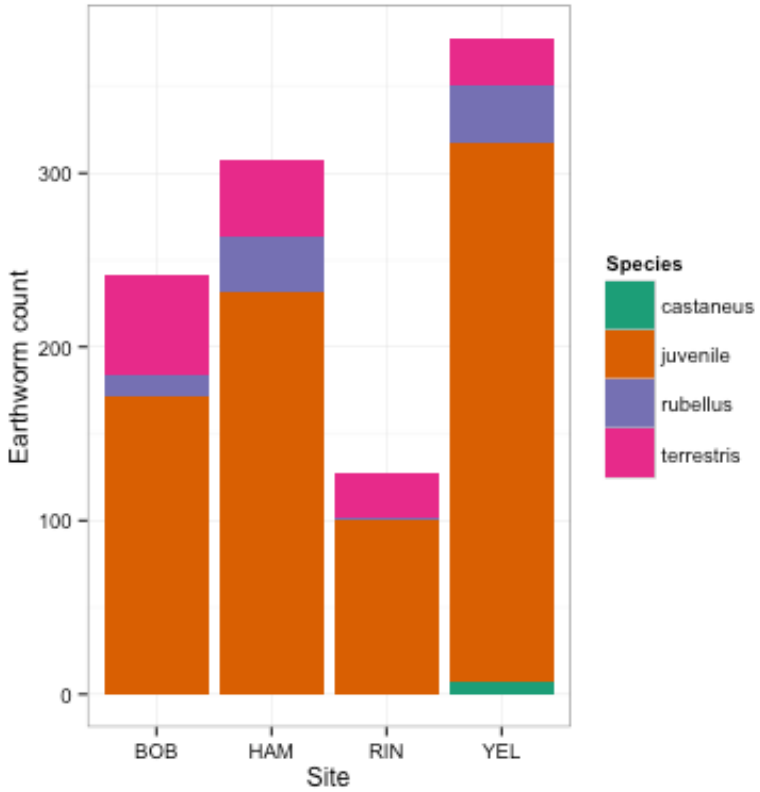
Appendix I.1. Total fresh earthworm biomass (g) for major earthworm genera at each of four forest sites. Data represent the sum of sampling 20 quadrats (0.25 m2) each over 3 years in each forest.



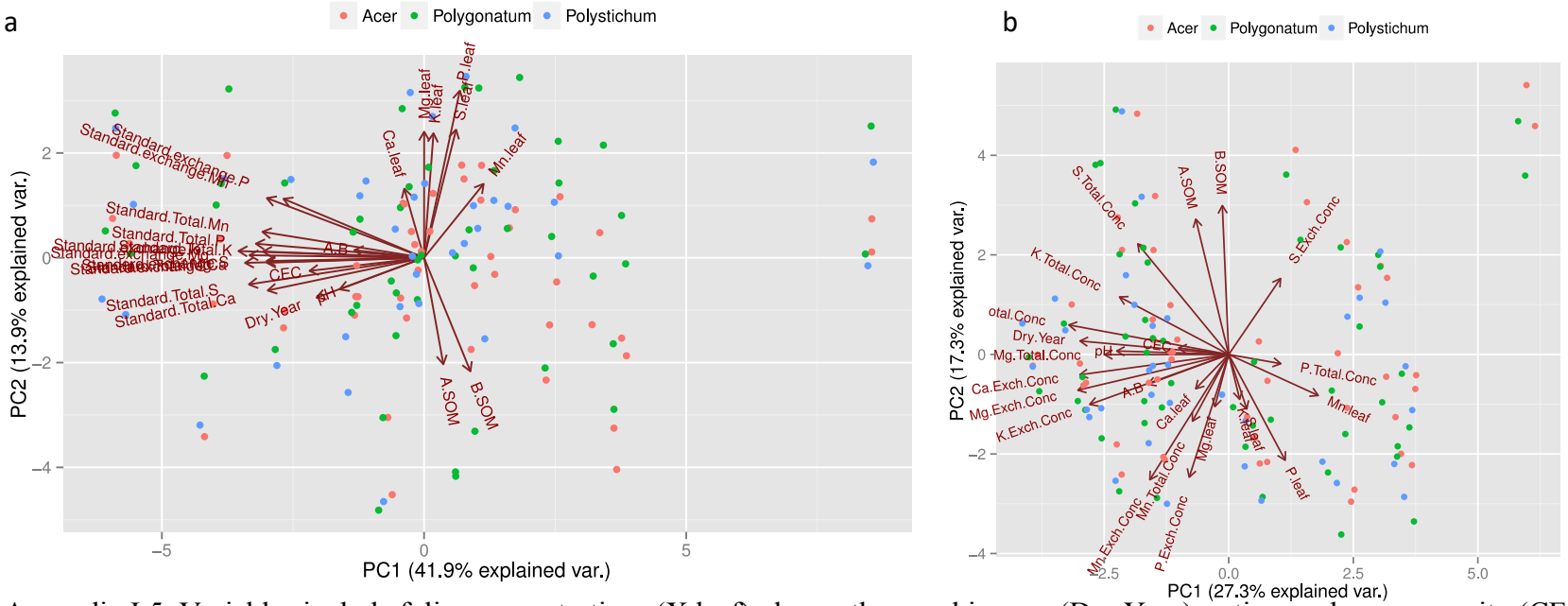
Appendices I.2 & I.3. Total (mg kg^{-1}), extractable (mg kg^{-1}) and extractable:total nutrient concentrations in the A horizon of four different forest sites as a function of mean annual dry earthworm biomass (g m^{-2}). See Appendix I.8 for summary statistics.



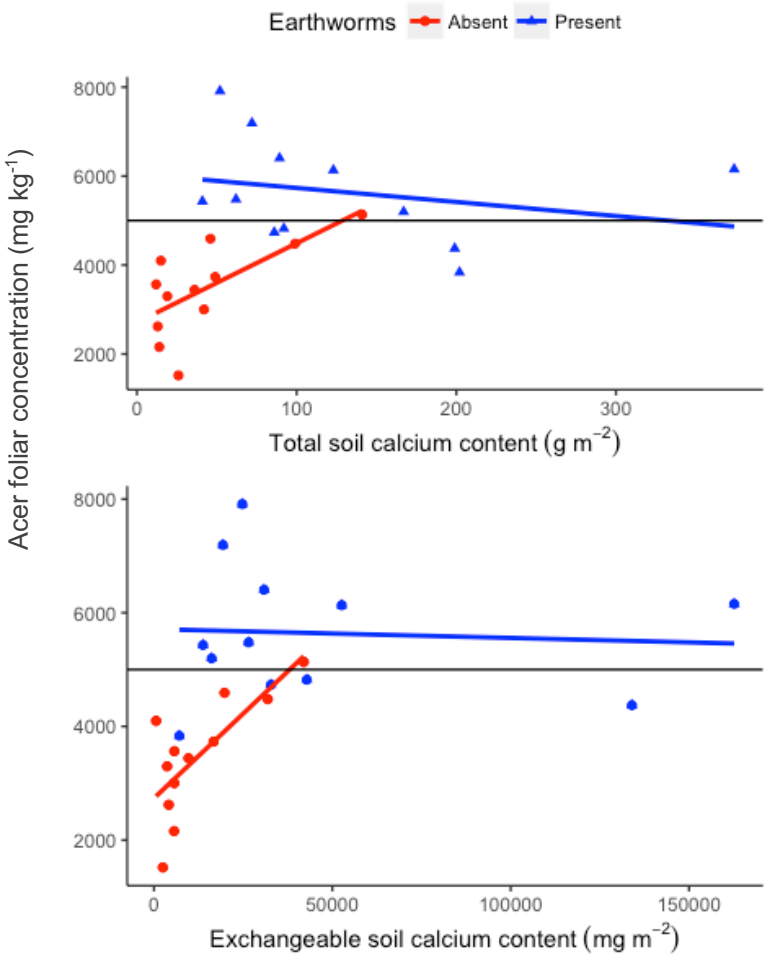
Appendix I.3. Total (mg kg^{-1}), extractable (mg kg^{-1}) and extractable:total nutrient concentrations in the B horizon of four different forest sites as a function of mean annual dry earthworm biomass (g m^{-2}). See Appendix I.8 for summary statistics.



Appendix I.4. Total earthworm abundance of *Lumbricus* species at each of four forests. Data represent the sum of sampling 20 quadrats (0.25 m^2) each over 3 years in each forest.



Appendix I.5. Variables include foliar concentrations (X.leaf), dry earthworm biomass (Dry.Year), cation exchange capacity (CEC), soil organic matter in A and B horizons (X.SOM), ratio of root volume in A:B horizons (A:B) with (a) total nutrient pools from 0 - 10cm (Standard.total.X), extractable nutrient pools from 0 - 10cm (Standard.exchange.X) and (b) total (X.Total.Conc) and extractable A horizon concentrations.



Appendix I.6. Relationship between tissue concentration (mg kg^{-1}) and soil calcium content standardized to 0-10 cm. Horizontal line represents minimum published range for healthy *Acer saccharum* trees grown in unfertilized forest conditions in July/August (Burton et al. 1993; Kolb and McCormick 1993).

Appendix I.7. Name, location and ecological properties of experimental sites.

Site	Location	Land use history	Leaf Area Index[1]	Soil suborder	pH	Dominant Trees
Bobolink Hill	42.382773, -76.246179	Passively reclaimed farmland (1940s)	5.12-6.87	Dystochepts	3.57 - 4.84	*Quercus* sp., *F. grandifolia*
Ringwood	42.445248, -76.367988	Timber harvest through early 1900s, not farmed	5.69-7.68	Fragiochepts/ Dystochepts	4.02 - 4.47	*Quercus* sp., *F. grandifolia*
Hammond Hill	42.416921, -76.302395	Passively reclaimed farmland (1940s)	4.55-7.44	Fragiochepts/ Dystochepts	3.89 - 5	*Quercus* sp., *Acer* sp., *F. grandifolia*
Yellow Barn	42.452632, -76.337764	Actively reforested farmland (~1933)	4.58-6.43	Fragiochepts	3.57 - 4.76	*Quercus* sp., *F. americana*

[1] LAI data from Dobson and Blossey (2015)

Appendix I.8. Summary statistics for linear mixed models of earthworm biomass on total, extractable and extractable: total soil nutrient concentration (mg kg $^{-1}$). All response variables (concentrations) are standardized to their respective means. Site is included as a random effect and each model contains 4 parameters (*k*).

		Intercept				Earthworm biomass					
		Estimate $^{\alpha}$	SE$^{\beta}$	t$^{\varphi}$	p$^{\delta}$	Estimate $^{\alpha}$	SE$^{\beta}$	t	p$^{\delta}$	R^2m^{ε}	R^2c^{χ}
Ca	Total A	0.44	0.25	1.78	0.076	0.42	0.11	3.79	0.0001	0.33	0.49
	Total B	0.5	0.1	4.89	0	0.11	0.021	5.32	0	0.56	0.72
	Extractable A	0.35	0.34	1.04	0.3	0.49	0.16	3.13	0.002	0.25	0.45
	Extractable B	0.94	0.29	3.24	0.0012	0.024	0.051	0.47	0.64	0.0066	0.63
	Total A:Extractable A	0.89	0.13	7.02	0	0.085	0.047	1.81	0.07	0.069	0.55
	Total B:Extractable B	6.43	1.92	3.35	0.00082	-1.65	0.39	-4.23	0	0.1	0.94
K	Total A	0.79	0.07	10.62	0	0.15	0.03	4.24	0.00022	0.39	0.51
	Total B	0.81	0.086	9.42	0	0.036	0.012	3.02	0.0025	0.14	0.81
	Extractable A	0.68	0.12	5.55	0	0.24	0.04	5	0	0.38	0.67
	Extractable B	1.25	0.26	4.76	0	-0.11	0.04	-2.81	0.005	0.15	0.77
	Total A:Extractable A	0.88	0.13	6.57	0	0.098	0.045	2.19	0.028	0.096	0.57
	Total B:Extractable B	1.52	0.29	5.33	0	-0.34	0.13	-2.55	0.0011	0.18	0.64
Mg	Total A	0.77	0.1	8	0	0.17	0.05	3.21	0.0013	0.31	0.31
	Total B	0.92	0.1	9.18	0	0.03	0.026	1.14	0.25	0.075	0.075

	Extractable A	0.46	0.23	1.98	0.047	0.41	0.1	3.98	0.00007	0.34	0.54
	Extractable B	0.86	0.26	3.25	0.0011	0.059	0.034	1.76	0.079	0.045	0.83
	Total A:Extractable A	0.74	0.2	3.7	0.0002	0.21	0.1	2.1	0.036	0.15	0.24
	Total B:Extractable B	0.15	0.059	2.5	0.013	0.00039	0.036	0.01	0.99	0	0.12
Mn	Total A	0.89	0.2	4.68	0	0.05	0.1	0.46	0.64	0.009	0.009
	Total B	0.9	0.13	6.89	0	0.025	0.022	1.12	0.26	0.041	0.58
	Extractable A	0.77	0.2	3.91	0.0001	0.15	0.08	1.96	0.05	0.09	0.52
	Extractable B	1.32	0.076	3.63	0.00028	-0.14	0.076	-1.86	0.063	0.13	0.5
	Total A:Extractable A	0.82	0.23	3.65	0.00026	0.14	0.1	1.38	0.17	0.069	0.17
	Total B:Extractable B	0.4	0.16	2.46	0.014	-0.11	0.097	-1.16	0.25	0.069	0.24
P	Total A	1.11	0.09	11.77	0	-0.1	0.04	-2.38	0.02	0.18	0.25
	Total B	1.08	0.11	9.56	0	-0.05	0.029	-1.61	0.11	0.14	0.14
	Extractable A	1.04	0.16	6.67	0	-0.02	0.07	-0.34	0.73	0.004	0.31
	Extractable B	1.73	0.5	3.48	0.0005	-0.26	0.1	-2.58	0.0097	0.21	0.58
	Total A:Extractable A	0.94	0.2	4.63	0	0.06	0.07	0.82	0.41	0.019	0.39
	Total B:Extractable B	0.093	0.03	3.15	0.0016	-0.034	0.017	-1.97	0.049	0.16	0.4
S	Total A	0.93	0.1	9.03	0	0.06	0.03	1.87	0.06	0.07	0.58
	Total B	0.87	0.063	13.83	0	0.033	0.012	2.63	0.0085	0.21	0.61

	Extractable A	1.1	0.13	8.43	0	-0.06	0.07	-0.9	0.37	0.034	0.034
	Extractable B	1.13	0.12	9.41	0	-0.046	0.03	-1.5	0.13	0.12	0.19
	Total A:Extractable A	1.18	0.14	8.2	0	-0.13	0.077	-1.68	0.093	0.11	0.11
	Total B:Extractable B	0.79	0.081	9.79	0	-0.11	0.041	-2.67	0.0076	0.28	0.39

α Standardized mixed model estimates calculated with maximum likelihood
β One standard error from the mean
φ t-values of maximum likelihood test
δ Estimated p-value based on a normal distribution
ε Marginal R^2: proportion of variance described by fixed effects only
χ Conditional R^2: proportion of variance described both fixed and random effects

Appendix I.9. Summary statistics for linear mixed models of elemental total (g m^{-2}) and extractable nutrient pools (g m^{-2}) in shallow (0 - 10 cm soil depth) and deep (10 - 20 cm soil depth) as a function of dry earthworm biomass (g m^{-2}). Response variables (nutrient pools) are log-transformed standardized (N = 24 plots). Site is included as a random effect and each model contains 4 parameters (*k*).

			Intercept				Earthworm biomass				R^2m^{ε}	R^2c^{χ}
			Estimate $^{\alpha}$	SE^{β}	t^{φ}	p^{δ}	Estimate $^{\alpha}$	SE^{β}	t^{φ}	p^{δ}		
Ca	Total	0 - 10cm	3.57	0.22	16.5	0	0.39	0.12	3.37	0.00074	0.33	0.33
		10 - 20cm	3.54	0.31	11.58	0	0.34	0.16	2.04	0.029	0.16	0.24
	Extractable	0 - 10cm	8.92	0.3	29.33	0	0.57	0.13	4.4	0.000011	0.36	0.6
		10 - 20cm	9.79	0.31	24.88	0	0.31	0.17	1.68	0.066	0.13	0.15
K	Total	0 - 10cm	3.37	0.17	19.55	0	0.2	0.09	2.13	0.033	0.16	0.16
		10 - 20cm	3.6	0.22	16.67	0	0.033	0.12	0.28	0.77	0.0036	0.017
	Extractable	0 - 10cm	8.27	0.23	35.48	0	0.39	0.11	3.26	0.0011	0.3	0.37
		10 - 20cm	9.06	0.21	32.46	0	0.045	0.11	-0.02	0.7	0.0066	0.0066
Mg	Total	0 - 10cm	4.41	0.29	15.34	0	0.44	0.15	2.86	0.0042	0.26	0.26
		10 - 20cm	5.47	0.23	22.18	0	0.074	0.12	0.61	0.55	0.015	0.015
	Extractable	0 - 10cm	7.63	0.31	24.98	0	0.63	0.13	5.03	0	0.41	0.65
		10 - 20cm	8.48	0.26	25.14	0	0.29	0.14	1.93	0	0.16	0.16
Mn	Total	0 - 10cm	3.78	0.32	11.83	0	0.32	0.17	1.89	0.059	0.13	0.13
		10 - 20cm	4.45	0.28	14.06	0	0.082	0.15	0.61	0.59	0.013	0.013
	Extractable	0 - 10cm	6.99	0.33	21.23	0	0.34	0.15	2.3	0.021	0.14	0.4
		10 - 20cm	7.99	0.24	21.4	0	-0.029	0.12	-0.87	0.81	0.0022	0.133
P	Total	0 - 10cm	3.87	0.23	16.91	0	0.12	0.12	0.96	0.34	0.039	0.039
		10 - 20cm	4.52	0.25	17.83	0	-0.12	0.09	-1.33	0.18	0.068	0.098
	Extractable	0 - 10cm	-1.73	0.33	-5.21	0	0.087	0.15	0.6	0.55	0.011	0.34
		10 - 20cm	6.34	0.34	12.66	0	-0.39	0.18	-3.1	0.002	0.16	0.22

S	Total	0 - 10cm	3.08	0.21	14.56	0	0.24	0.11	2.13	0.033	0.16	0.21
		10 - 20cm	3.31	0.24	13.66	0	0.05	0.1	0.47	0.64	0.008	0.19
	Extractable	0 - 10cm	7.5	0.27	28.06	0	0.17	0.14	1.17	0.24	0.056	0.056
		10 - 20cm	8.44	0.23	36.71	0	-0.12	0.08	-1.49	0.14	0.07	0.13

α Standardized mixed model estimates calculated with maximum likelihood

β One standard error from the mean

φ t-values of maximum likelihood test

δ Estimated p-value based on a normal distribution

ε Marginal R^2: proportion of variance described by fixed effects only

χ Conditional R^2: proportion of variance described both fixed and random effects

Appendix I.10. Summary statistics for linear mixed models of relationship between plant leaf tissue (mg kg^{-1}) and earthworm biomass (g m^{-2}), with site as a random effect and each model contains 4 parameters (*k*).

Nutrient	Tissue	Species	Intercept				Earthworm biomass				R^2m^{ε}	R^2c^{χ}
			Estimate $^{\alpha}$	SE$^{\beta}$	t$^{\varphi}$	p$^{\delta}$	Estimate $^{\alpha}$	SE$^{\beta}$	t$^{\varphi}$	p$^{\delta}$		
Ca	Leaves	*A. saccharum*	3594	351	10.22	0	775	165	4.7	0	0.47	0.52
		P. pubescens	6015	566	10.62	0	-24	302	-0.08	0.94	0.0003	0.0042
		P. acrostichoides	1640	270	6.06	0	318	126	2.53	0.011	0.27	0.27
	Roots	*A. saccharum*	1629	288	5.66	0	586	154	3.8	0.00015	0.39	0.39
		P. pubescens	2921	424	6.89	0	-160	216	-0.74	0.46	0.022	0.022
		P. acrostichoides	2153	255	8.45	0	588	111	5.3	0	0.64	0.7
K	Leaves	*A. saccharum*	4446	275	16.16	0	-3	146	-0.02	0.98	0	0.0076
		P. pubescens	20250	1349	15.02	0	-862	554	-1.55	0.12	0.082	0.25
		P. acrostichoides	14099	1041	13.54	0	-187	484	-0.39	0.7	0.009	0.009
	Roots	*A. saccharum*	4135	437	9.46	0	47.89	227	0.21	0.83	0.002	0.023
		P. pubescens	3492	630	5.53	0	181	293	0.62	0.54	0.017	0.096
		P. acrostichoides	3641	386	9.44	0	-514	178	-2.89	0.0038	0.35	0.42
Mg	Leaves	*A. saccharum*	1092	69	15.81	0	60	37	1.63	0.1	0.1	0.1
		P. pubescens	1798	206	8.73	0	31	77	0.41	0.68	0.0056	0.25
		P. acrostichoides	2202	161	13.67	0	110	68	1.61	0.11	0.12	0.2
	Roots	*A. saccharum*	610	67	9.16	0	55	30	1.86	0.063	0.13	0.25
		P. pubescens	743	53	14.14	0	5	27	0.18	0.86	0.0014	0.0014
		P. acrostichoides	951	92	10.32	0	-36	45	-0.79	0.42	0.04	0.046

Mn	Leaves	*A. saccharum*	1030	130	7.93	0	-176	58	-3.05	0.0023	0.27	0.36
		P. pubescens	535	105	5.09	0	-132	32	-4.14	0.000035	0.32	0.59
		P. acrostichoides	157	20	7.69	0	-18	7	-2.58	0.0099	0.22	0.47
	Roots	*A. saccharum*	456	53	8.67	0	-58	28	-2.05	0.04	0.16	0.16
		P. pubescens	230	31	7.46	0	-59	16	-3.77	0.00016	0.37	0.37
		P. acrostichoides	367	90	4.05	0	-73	44	-1.65	0.1	0.15	0.15
P	Leaves	*A. saccharum*	990	135	7.31	0	-22	35	-0.62	0.54	0.0087	0.51
		P. pubescens	1629	212	7.69	0	-115	48	-2.38	0.017	0.096	0.63
		P. acrostichoides	1685	152	11.08	0	-133	71	-1.88	0.061	0.17	0.17
	Roots	*A. saccharum*	1164	129	9.04	0	-142	69	-2.05	0.04	0.15	0.15
		P. pubescens	972	91	10.67	0	-77	38	-2	0.046	0.15	0.31
		P. acrostichoides	938	84	11.19	0	-147	41	-3.57	0.00035	0.46	0.46
S	Leaves	*A. saccharum*	770	56	13.66	0	33	30	1.08	0.28	0.049	0.049
		P. pubescens	974	75	13.04	0	-3	21	-0.13	0.9	0.00042	0.44
		P. acrostichoides	1257	96	13.16	0	21	44	0.46	0.64	0.01	0.01
	Roots	*A. saccharum*	699	56	12.45	0	57	30	1.88	0.06	0.13	0.13
		P. pubescens	808	81	9.97	0	-52	30	-1.75	0.081	0.11	0.38
		P. acrostichoides	954	75	12.8	0	-122	37	-3.35	0.00081	0.43	0.43

α Standardized mixed model estimates calculated with maximum likelihood

β One standard error from the mean

φ t-values of maximum likelihood test

δ Estimated p-value based on a normal distribution

ε Marginal R^2: proportion of variance described by fixed effects only

χ Conditional R^2: proportion of variance described both fixed and random effects

Appendix I.11. Summary statistics for linear mixed models foliar Ca in *A. saccharum*. Fixed effects include dry earthworm biomass (g m^{-2}) and total or extractable soil Ca in the top 0 – 10cm, and site was a random effect. The earthworm biomass* extractable soil Ca interaction because it was not significant.

		Estimate $^{\alpha}$	SE$^{\beta}$	t$^{\varphi}$	p$^{\delta}$
Total Ca	Intercept	720	1325	0.54	0.59
	Earthworm biomass	8185	2590	3.16	0.0016
	log(Ca)	801	376	2.13	0.033
	Earthworm biomass*log(Ca)	-1501	604	-2.48	0.013
	R^2m^{ε} 0.54				
	R^2c^{χ} 0.54				
	k^{η} 6				
Extractable Ca	Intercept	480	1978	0.24	0.81
	Earthworm biomass	337	220	1.53	0.13
	log(Ca)	1669	549	3.08	0.0024
	R^2m^{ε} 0.54				
	R^2c^{χ} 0.54				
	k^{η} 5				

α Standardized mixed model estimates calculated with maximum likelihood

β One standard error from the mean

φ t-values of maximum likelihood test

δ Estimated p-value based on a normal distribution

ε Marginal R^2: proportion of variance described by fixed effects only

χ Conditional R^2: proportion of variance described both fixed and random effects

η Number of parameters (k) in the model

Appendix I.12. Summary statistics for linear mixed models of root biomass in soil monoliths. Fixed effects include dry earthworm biomass (g m^{-2}), horizon, and their interaction. Site is a random effect and the model contains 5 parameters (*k*).

	Estimate $^{\alpha}$	SE$^{\beta}$	t$^{\varphi}$	p$^{\delta}$
Intercept	0.7	0.01	5.16	0
Horizon (B)	-0.06	0.01	-3.68	0
Earthworm presence (True)	-0.04	0.02	-2.69	0.01
Earthworm presence*Horizon	0.07	0.02	3.07	0

α Standardized mixed model estimates calculated with maximum likelihood

β One standard error from the mean

φ t-values of maximum likelihood test

δ Estimated p-value based on a normal distribution

Appendix I.13. Random effect of site in linear mixed models of total, extractable and extractable: total soil nutrient concentration (mg kg $^{-1}$).

		Site		Residual	
		Variance	Standard Dev	Variance	Standard Dev
Ca	Total A	0.12	0.34	0.62	0.79
	Total B	0.05	0.22	0.06	0.35
	Extractable A	0.33	0.38	0.5	0.17
	Extractable B	0.79	0.89	0.28	0.53
	Total A:Extractable A	0.05	0.23	0.16	0.39
	Total B:Extractable B	25.94	5.1	11.19	3.35
K	Total A	0.02	0.15	0.05	0.22
	Total B	0.04	0.2	0.05	0.22
	Extractable A	0	0	0.19	0.43
	Extractable B	0.73	0.85	0.11	0.34
	Total A:Extractable A	0.03	0.17	0.16	0.4
	Total B:Extractable B	0.79	0.89	0.23	0.48
Mg	Total A	0	0	13	0.36
	Total B	0	0	0.11	0.33
	Extractable A	0.05	0.23	0.64	0.8
	Extractable B	0	0	0.11	0.33
	Total A:Extractable A	0.13	0.36	0.41	0.64
	Total B:Extractable B	0.03	0.16	0.02	0.14
Mn	Total A	0.91	0.21	0.03	0.1
	Total B	0.08	0.28	0.1	0.31
	Extractable A	0.14	0.37	0.38	0.61
	Extractable B	1.05	1.03	0.53	0.73
	Total A:Extractable A	0	0	0.51	0.72
	Total B:Extractable B	0.16	0.4	0.18	0.42
P	Total A	0.01	0.11	0.08	0.28
	Total B	0	0	0.14	0.38

	Extractable A	0.12	0.34	0.22	0.46
	Extractable B	2.16	1.47	0.93	0.96
	Total A:Extractable A	0.08	0.29	0.28	0.53
	Total B:Extractable B	0.01	0.09	0	0.06
S	Total A	0.93	0.12	0.07	0.04
	Total B	0.04	0.2	0.01	0.12
	Extractable A	0	0	0.23	0.48
	Extractable B	0.07	0.27	0.1	0.32
	Total A:Extractable A	0	0	0.28	0.53
	Total B:Extractable B	0	0	0.05	0.22

Appendix I.14. The random effect of site in linear mixed models of total and extractable nutrient content (g m^{-2}).

			Intercept (Site)		Residual	
			Variance	Standard Dev	Variance	Standard Dev
Ca	Total	0 - 10cm	0.15	0.38	0.51	0.71
		10 - 20cm	0.01	0.01	1.23	1.11
	Extractable	0 - 10cm	0.34	0.58	0.89	0.94
		10 - 20cm	9.85	0.39	1.08	1.04
K	Total	0 - 10cm	0.01	0.12	0.39	0.62
		10 - 20cm	0	0	0.63	0.79
	Extractable	0 - 10cm	0.21	0.45	0.55	0.74
		10 - 20cm	0.17	0.42	0.47	0.69
Mg	Total	0 - 10cm	0.01	0.09	1.1	1.05
		10 - 20cm	0.04	0.2	0.68	0.82
	Extractable	0 - 10cm	0.54	0.74	0.74	0.86
		10 - 20cm	0.26	0.51	0.68	0.82
Mn	Total	0 - 10cm	0.1	0.32	1.29	1.14
		10 - 20cm	0.1	0.31	1	1
	Extractable	0 - 10cm	0.56	0.75	0.92	0.96
		10 - 20cm	0.44	0.66	0.43	0.66
P	Total	0 - 10cm	0.04	0.2	0.67	0.82
		10 - 20cm	0.14	0.38	0.38	0.62
	Extractable	0 - 10cm	0.78	0.88	0.78	0.88
		10 - 20cm	0.73	0.85	0.99	0.99
S	Total	0 - 10cm	0.14	0.37	0.49	0.7
		10 - 20cm	0.1	0.32	0.44	0.66
	Extractable	0 - 10cm	0.01	0.1	0.95	0.98
		10 - 20cm	0.12	0.35	0.3	0.55

Appendix I.15. Random effect of site in linear mixed models of leaf and root tissue nutrient concentration (mg kg $^{-1}$).

			Intercept (Site)		Residual	
			Variance	Standard Dev	Variance	Standard Dev
Ca	Leaves	*A. saccharum*	121935	350	1239012	1113
		P. pubescens	16818	130	4244073	2060
		P. acrostichoides	0	0	550551	742
	Roots	*A. saccharum*	0	0	1111423	1054
		P. pubescens	0	0	2428751	1558
		P. acrostichoides	72330	269	331903	576
K	Leaves	*A. saccharum*	7604	87	988941	995
		P. pubescens	3084291	1759	13839643	3720
		P. acrostichoides	0	0	8166151	2858
	Roots	*A. saccharum*	52613	2239	2341338	1530
		P. pubescens	333550	578	3797390	1949
		P. acrostichoides	100373	317	885754	941
Mg	Leaves	*A. saccharum*	0	0	64012	253
		P. pubescens	88345	297	267726	517
		P. acrostichoides	15791	126	159380	399
	Roots	*A. saccharum*	5800	76	36756	191
		P. pubescens	0	0	37300	193
		P. acrostichoides	368	19	61479	248
Mn	Leaves	*A. saccharum*	22211	149	149852	387
		P. pubescens	30251	174	45257	213
		P. acrostichoides	759	28	1574	40
	Roots	*A. saccharum*	0	0	37117	193
		P. pubescens	0	0	12800	113
		P. acrostichoides	0	0	60085	245
P	Leaves	*A. saccharum*	56382	237	55300	235
		P. pubescens	14136	385	102521	320
		P. acrostichoides	0	0	174105	417
	Roots	*A. saccharum*	0	0	222369	472
		P. pubescens	12581	112	57685	240
		P. acrostichoides	114	11	51322	227
S	Leaves	*A. saccharum*	0	0	42699	207
		P. pubescens	16115	127	20394	143
		P. acrostichoides	0	0	68685	262

	Roots	*A. saccharum*	0	0	42274	206
		P. pubescens	13896	118	32369	180
		P. acrostichoides	0	0	40773	202

Appendix I.16. Random effect of site in linear mixed models of foliar Ca concentration (mg kg $^{-1}$) and root biomass (g).

	Intercept (Site)		Residual	
	Variance	Standard Dev	Variance	Standard Dev
Total Ca	0	0	1021642	1011
Extractable Ca	0	0	1169480	1081
Root biomass	45.57	6.75	422.42	20.55

APPENDIX II

Appendix II.1. Individual model fits of biomass models using random and fixed effects (R^2_c) and fixed effects only (R^2_m). Soil nutrients are measured as concentrations (w/w) in the A horizon.

Species		R^2_m	R^2_c	n
Actaea	Biomass	0.2	0.53	33
	Mycorrhizae	0.22	0.32	35
	Fine root	0.053	0.15	69
	Total N	0.2	0.7	48
	Total P	0.59	0.63	64
	Extractable P	0.014	0.2	64
Aquilegia	Biomass	0.25	0.74	16
	Mycorrhizae	0.075	0.24	27
	Fine root	0.19	0.31	37
	Total N	0.19	0.69	36
	Total P	0.59	0.62	48
	Extractable P	0.014	0.18	48
Cornus	Biomass	0.08	0.08	18
	Mycorrhizae	0.011	0.13	19
	Fine root	0.1	0.18	36
	Total N	0.2	0.7	48
	Total P	0.59	0.63	64
	Extractable P	0.014	0.2	64
Prenanthes	Biomass	0.23	0.23	18
	Mycorrhizae	0.084	0.084	19
	Fine root	0.002	0.002	36
	Total N	0.2	0.7	48
	Total P	0.59	0.63	64
	Extractable P	0.014	0.2	64
Quercus	Biomass	0.037	0.31	35
	Mycorrhizae	0.081	0.09	57
	Fine root	0.036	0.18	59
	Total N	0.2	0.71	60
	Total P	0.6	0.63	80
	Extractable P	0.014	0.22	80

Appendix II.2. Fixed model components for the effects of deer and earthworms on biomass of *Actaea pachypoda* (Fisher's C = 8.15; df = 10; p=0.61; AIC = 84.15; AICc = -409.85; k=38; n=33). Soil nutrients are measured as concentrations (w/w) in the A horizon. Significant paths are bolded.

Response	Predictor	Estimate	SE	p
Biomass	**Worm**	**-1.014**	**0.41**	**0.0225**
Biomass	Mycorrhizae	-0.18	0.16	0.27
Biomass	Total P	-0.28	0.29	0.34
Biomass	Extractable P	-0.28	0.29	0.35
Biomass	Fine root	-0.12	0.16	0.48
Biomass	Total N	0.22	0.3	0.48
Biomass	Height	-0.05	0.14	0.72
Biomass	Deer	-0.071	0.53	0.89
Mycorrhizae	**Extractable P**	**-0.63**	**0.22**	**0.0079**
Mycorrhizae	Deer	-0.38	0.43	0.38
Mycorrhizae	Worm	0.0093	0.35	0.98
Fine root	**Worm**	**-0.41**	**0.23**	**0.078**
Fine root	Deer	-0.24	0.23	0.3
Total N	**Deer**	**-1.08**	**0.22**	**0**
Total N	Worm	0.095	0.2	0.63
Total P	**Deer**	**-1.24**	**0.15**	**0**
Total P	**Worm**	**-0.92**	**0.15**	**0**
Extractable P	Deer	0.22	0.23	0.34
Extractable P	Worm	-0.11	0.23	0.64

Appendix II.3. Fixed model components for the effects of deer and earthworms on biomass of *Aquilegia canadense* (Fisher's C = 8.31; df = 10; p=0.60; AIC = 80.31; AICc = -46.55; k=36; n=16). Soil nutrients are measured as concentrations (w/w) in the A horizon. Significant paths are bolded.

Response	predictor	Estimate	SE	p
Biomass	Total N	0.84	0.6	0.22
Biomass	Deer	0.94	0.87	0.33
Biomass	Worm	0.66	0.62	0.33
Biomass	Height	0.21	0.21	0.35
Biomass	Fine root	-0.42	0.45	0.39
Biomass	Extractable P	0.36	0.64	0.6
Biomass	Mycorrhizae	0.024	0.45	0.96
Mycorrhizae	Worm	0.31	0.37	0.4
Mycorrhizae	Deer	-0.38	0.47	0.42
Fine root	**Worm**	**0.75**	**0.29**	**0.014**
Fine root	**Deer**	**0.53**	**0.29**	**0.075**
Total N	**Deer**	**-1.07**	**0.25**	**0.0002**
Total N	Worm	0.1	0.23	0.68
Total P	**Deer**	**-1.24**	**0.18**	**0**
Total P	**Worm**	**-0.92**	**0.18**	**0**
Extractable P	Deer	0.22	0.27	0.42
Extractable P	Worm	-0.11	0.27	0.69

Appendix II.4. Fixed model components for the effects of deer and earthworms on biomass of *Cornus racemosa* (Fisher's C = 11.25; df = 12; p = 0.51; AIC = 81.25; AICc = 278.75; k = 35; n = 29). Soil nutrients are measured as concentrations (w/w) in the A horizon. Significant paths are bolded.

Response	predictor	Estimate	SE	p
Biomass	Deer	-0.7	0.6	0.26
Biomass	Extractable P	-0.22	0.35	0.54
Biomass	Fine root	-0.18	0.35	0.61
Biomass	Total N	-0.11	0.31	0.74
Biomass	Mycorrhizae	-0.084	0.32	0.8
Biomass	Height	-0.051	0.28	0.86
Biomass	Worm	-0.1	0.59	0.87
Mycorrhizae	Worm	-0.46	0.33	0.17
Mycorrhizae	Deer	-0.16	0.46	0.74
Fine root	**Worm**	**-0.56**	**0.22**	**0.013**
Fine root	Deer	0.27	0.22	0.23
Total N	**Deer**	**-1.08**	**0.22**	**0**
Total N	Worm	0.095	0.2	0.63
Total P	**Deer**	**-1.24**	**0.15**	**0**
Total P	**Worm**	**-0.92**	**0.15**	**0**
Extractable P	Deer	0.22	0.23	0.34
Extractable P	Worm	-0.11	0.23	0.64

Appendix II.5. Fixed model components for the effects of deer and earthworms on biomass of *Prenanthes alba* (Fisher's C = 11.72; df = 12; p = 0.47; AIC = 85.72; AICc = -54.88; k = 37; n = 18). Soil nutrients are measured as concentrations (w/w) in the A horizon. Significant paths are bolded.

Response	predictor	Estimate	SE	p
Biomass	Height	0.35	0.18	0.092
Biomass	Fine root	0.32	0.22	0.18
Biomass	Extractable P	0.18	0.23	0.45
Biomass	Deer	0.23	0.39	0.58
Biomass	Mycorrhizae	-0.067	0.2	0.74
Biomass	Total N	0.071	0.26	0.79
Biomass	Worm	-0.069	0.46	0.88
Mycorrhizae	Worm	-0.49	0.59	0.43
Mycorrhizae	Deer	-0.4	0.53	0.47
Fine root	Worm	0.075	0.34	0.82
Fine root	Deer	0.032	0.35	0.92
Total N	**Deer**	**-1.08**	**0.22**	**0**
Total N	Worm	0.095	0.2	0.63
Total P	**Deer**	**-1.24**	**0.15**	**0**
Total P	**Worm**	**-0.92**	**0.15**	**0**
Extractable P	Deer	0.22	0.23	0.34
Extractable P	Worm	0.11	0.23	0.64

Appendix II.6. Fixed model components for the effects of deer and earthworms on biomass of *Quercus rubra* (Fisher's C = 5.43; df = 10; p = 0.86; AIC = 77.43; AICc = -1254.57; k = 36; n =35). Soil nutrients are measured as concentrations (w/w) in the A horizon. Significant paths are bolded.

Response	predictor	estimate	SE	p
Biomass	Total N	-0.14	0.23	0.55
Biomass	Mycorrhizae	-0.18	0.31	0.56
Biomass	Height	0.1	0.21	0.64
Biomass	Deer	0.25	0.6	0.67
Biomass	Fine root	-0.06	0.2	0.77
Biomass	Extractable P	0.067	0.33	0.84
Biomass	Total P	0.045	0.47	0.93
Biomass	Worm	0.002	0.32	1
Mycorrhizae	**Worm**	**-0.54**	**0.26**	**0.046**
Mycorrhizae	Deer	0.22	0.26	0.4
Fine root	Worm	-0.39	0.25	0.12
Fine root	Deer	-0.016	0.25	0.95
Total N	**Deer**	**-1.09**	**0.19**	**0**
Total N	Worm	0.094	0.17	0.59
Total P	**Deer**	**-1.25**	**0.14**	**0**
Total P	**Worm**	**-0.92**	**0.14**	**0**
Extractable P	Deer	0.22	0.21	0.28
Extractable P	Worm	-0.12	0.21	0.6

Appendix II.7. Standard deviation of the random effect of site on all species on dry plant biomass, calculated using maximum likelihood. Soil nutrients are measured as concentrations (w/w) in the A horizon.

Species	Variable	Site	
		Intercept	Residual
Actaea	Biomass	0.36	0.42
	Mycorrhizae	3.7	9.84
	Fine root	0.028	0.082
	Total N	0.1	0.075
	Total P	0.044	0.15
	Extractable P	0.19	0.39
Aquilegia	Biomass	0.21	0.15
	Mycorrhizae	4.64	10.1
	Fine root	0.029	0.7
	Total N	0.1	0.08
	Total P	0.038	0.15
	Extractable P	0.18	0.29
Cornus	Biomass	0	0.03
	Mycorrhizae	0	15.12
	Fine root	0	0.14
	Total N	0.1	0.075
	Total P	0.044	0.15
	Extractable P	0.19	0.39
Prenanthes	Biomass	0	0.03
	Mycorrhizae	0	15.12
	Fine root	0	0.14
	Total N	0.1	0.075
	Total P	0.044	0.15
	Extractable P	0.19	0.39
Quercus	Biomass	0.17	0.27
	Mycorrhizae	1.04	9.8
	Fine root	0.034	0.082
	Total N	0.1	0.074
	Total P	0.047	0.14
	Extractable P	0.19	0.38

Appendix II.8. Correlated errors of fixed variables from dry plant biomass models. Soil nutrients are measured as concentrations (w/w) in the A horizon.

		Extractable P	Total P	Mycorrhizae	Total N
Actaea	Extractable P	-			
	Total P	NS	-		
	Mycorrhizae	NS	NS	-	
	Total N	NS	NS	NS	-
	Fine root	NS	0.16¥	NS	NS
Aquelegia	Extractable P	-			
	Total P	NS	-		
	Mycorrhizae	NS	NS	-	
	Total N	NS	NS	0.2¥	-
	Fine root	NS	0.19¥	NS	NS
Cornus	Extractable P	-			
	Total P	NS	-		
	Mycorrhizae	NS	NS	-	
	Total N	NS	NS	0.24*	-
	Fine root	NS	NS	NS	NS
Prenanthes	Extractable P	-			
	Total P	NS	-		
	Mycorrhizae	NS	NS	-	
	Total N	NS	NS	0.15¥	-
	Fine root	NS	NS	0.22*	0.37***
Quercus	Extractable P	-			
	Total P	NS	-		
	Mycorrhizae	NS	NS	-	
	Total N	NS	NS	NS	-
	Fine root	NS	NS	NS	NS

Appendix II.9. Individual model fits for survival models using random and fixed effects (R^2_c) and fixed effects only (R^2_m). Soil nutrients are measured as concentrations (w/w) in the A horizon.

Species		R^2_m	R^2_c	n
Actaea	Survival	0.44	0.69	33
	Mycorrhizae	0.14	0.28	43
	Fine root	0.05	0.15	69
	Total N	0.2	0.7	48
	Total P	0.59	0.63	64
	Extractable P	0.01	0.2	64
Aquilegia	Survival	0.6	0.86	17
	Mycorrhizae	0.01	0.2	46
	Fine root	0.19	0.31	37
	Total N	0.19	0.69	36
	Total P	0.59	0.62	48
	Extractable P	0.01	0.18	48
Cornus	Survival	0.41	0.96	29
	Mycorrhizae	0.01	0.13	51
	Fine root	0.1	0.18	71
	Total N	0.2	0.7	48
	Total P	0.59	0.63	64
	Extractable P	0.01	0.2	64
Prenanthes	Survival	0.91	0.91	18
	Mycorrhizae	0.02	0.07	36
	Fine root	0.01	0.01	36
	Total N	0.2	0.7	48
	Total P	0.59	0.63	64
	Extractable P	0.01	0.2	64
Quercus	Survival	0.47	0.95	36
	Mycorrhizae	0.08	0.09	57
	Fine root	0.04	0.17	59
	Total N	0.2	0.71	60
	Total P	0.59	0.63	80
	Extractable P	0.01	0.22	80

Appendix II.10. Fixed model components for the effects of deer and earthworms on survival of *Actaea pachypoda* (Fisher's C = 7.49; df = 10; p=0.68; AIC = 81.49; AICc = -480.91; k=37; n=33). Soil nutrients are measured as concentrations (w/w) in the A horizon. Significant paths are bolded.

Response	Predictor	estimate	SE	p
Survival	**Extractable P**	**-0.51**	**0.2**	**0.02**
Survival	Total P	0.32	0.2	0.11
Survival	Fine root	0.15	0.11	0.16
Survival	Mycorrhizae	-0.15	0.1	0.17
Survival	Worm	-0.32	0.27	0.26
Survival	Total N	0.08	0.21	0.71
Survival	Height	0.03	0.09	0.78
Survival	Deer	-0.1	0.35	0.78
Mycorrhizae	**Extractable P**	**-0.37**	**0.15**	**0.02**
Mycorrhizae	Deer	-0.04	0.29	0.88
Mycorrhizae	Worm	0.04	0.3	0.89
Fine root	**Worm**	**-0.41**	**0.23**	**0.08**
Fine root	Deer	-0.23	0.23	0.3
Total N	**Deer**	**-1.08**	**0.22**	**0**
Total N	Worm	0.09	0.2	0.63
Total P	**Deer**	**-1.24**	**0.15**	**0**
Total P	**Worm**	**-0.92**	**0.15**	**0**
Extractable P	Deer	0.22	0.23	0.34
Extractable P	Worm	-0.12	0.23	0.64

Appendix II.11. Fixed model components for the effects of deer and earthworms on survival of *Aquilegia canadense* (Fisher's C = 8.31; df = 10; p=0.6; AIC = 80.31; AICc = -52.89; k=36; n=17). Soil nutrients are measured as concentrations (w/w) in the A horizon. Significant paths are bolded.

Response	predictor	estimate	SE	p
Biomass	**Total P**	**-0.56**	**0.17**	**0.02**
Biomass	**Extractable P**	**-0.31**	**0.14**	**0.08**
Biomass	Worm	-0.2	0.22	0.41
Biomass	Deer	-0.29	0.35	0.44
Biomass	Height	0.02	0.05	0.72
Biomass	Total N	-0.06	0.15	0.73
Biomass	Mycorrhizae	-0.02	0.09	0.86
Biomass	Fine root	-0.01	0.11	0.97
Mycorrhizae	Worm	0.2	0.27	0.46
Mycorrhizae	Deer	0.12	0.27	0.65
Fine root	**Worm**	**0.75**	**0.29**	**0.01**
Fine root	**Deer**	**0.53**	**0.29**	**0.07**
Total N	**Deer**	**-1.07**	**0.25**	**0**
Total N	Worm	0.1	0.23	0.68
Total P	**Deer**	**-1.24**	**0.18**	**0**
Total P	**Worm**	**-0.91**	**0.18**	**0**
Extractable P	Deer	0.22	0.27	0.42
Extractable P	Worm	-0.11	0.27	0.69

Appendix II.12. Fixed model components for the effects of deer and earthworms on survival of *Cornus racemosa* (Fisher's C = 13.79; df = 12; p = 0.31; AIC = 83.79; AICc = 276.21; k = 35; n = 29). Soil nutrients are measured as concentrations (w/w) in the A horizon. Significant paths are bolded.

Response	predictor	estimate	SE	p
Survival	**Extractable P**	**-0.51**	**0.08**	**0**
Survival	**Deer**	**-0.55**	**0.15**	**0.002**
Survival	**Worm**	**0.23**	**0.07**	**0.005**
Survival	**Total N**	**-0.3**	**0.1**	**0.007**
Survival	Mycorrhizae	-0.06	0.04	0.18
Survival	Height	-0.01	0.03	0.67
Survival	Fine root	-0.02	0.04	0.7
Mycorrhizae	Deer	0.16	0.27	0.56
Mycorrhizae	Worm	0.12	0.27	0.65
Fine root	**Deer**	**-0.56**	**0.22**	**0.01**
Fine root	Worm	0.27	0.22	0.23
Total N	**Deer**	**-1.08**	**0.22**	**0**
Total N	Worm	0.09	0.2	0.63
Total P	**Deer**	**-1.24**	**0.15**	**0**
Total P	**Worm**	**-0.92**	**0.15**	**0**
Extractable P	Deer	0.22	0.23	0.345
Extractable P	Worm	**-0.11**	0.23	0.64

Appendix II.13. Fixed model components for the effects of deer and earthworms on survival of *Prenanthes alba* (Fisher's C = 10.82; df = 10; p = 0.37; AIC = 82.82; AICc = -57.4; k = 36; n = 18). Soil nutrients are measured as concentrations (w/w) in the A horizon. Significant paths are bolded.

Response	predictor	estimate	SE	p
Survival	**Extractable P**	**-0.62**	**0.09**	**0.004**
Survival	**Worm**	**-1.05**	**0.33**	**0.02**
Survival	**Total P**	**-0.55**	**0.22**	**0.05**
Survival	Total N	-0.13	0.17	0.46
Survival	Deer	-0.14	0.27	0.62
Survival	Mycorrhizae	-0.03	0.08	0.75
Survival	Fine root	0.02	0.07	0.84
Survival	Height	0.01	0.35	0.94
Mycorrhizae	Deer	-0.24	0.33	0.51
Mycorrhizae	Worm	-0.13	0.35	0.71
Fine root	Worm	0.08	0.34	0.83
Fine root	Deer	0.03	0.35	0.92
Total N	**Deer**	**-1.08**	**0.22**	**0**
Total N	Worm	0.09	0.2	0.63
Total P	**Deer**	**-1.24**	**0.15**	**0**
Total P	**Worm**	**-0.92**	**0.15**	**0**
Extractable P	Deer	0.22	0.23	0.34
Extractable P	Worm	-0.11	0.23	0.64

Appendix II.14. Fixed model components for the effects of deer and earthworms on survival of *Quercus rubra* (Fisher's C = 5.43; df = 10; p = 0.86; AIC = 77.43; AICc = -2386.57; k = 36; n =36). Soil nutrients are measured as concentrations (w/w) in the A horizon. Significant paths are bolded.

Response	predictor	estimate	SE	p
Survival	**Worm**	**-1.13**	**0.17**	**0**
Survival	**Total P**	**-0.46**	**0.13**	**0.001**
Survival	**Extractable P**	**0.62**	**0.18**	**0.003**
Survival	**Total N**	**0.65**	**0.2**	**0.003**
Survival	**Mycorrhizae**	**0.17**	**0.08**	**0.04**
Survival	Deer	0.51	0.34	0.15
Survival	Height	-0.11	0.08	0.17
Survival	Fine root	0.1	0.07	0.19
Mycorrhizae	**Worm**	**-0.53**	**0.26**	**0.05**
Mycorrhizae	Deer	0.22	0.26	0.4
Fine root	Worm	-0.39	0.25	0.12
Fine root	Deer	-0.02	0.25	0.96
Total N	**Deer**	**-1.09**	**0.19**	**0**
Total N	Worm	0.09	0.17	0.6
Total P	**Deer**	**-1.25**	**0.14**	**0**
Total P	**Worm**	**-0.92**	**0.14**	**0**
Extractable P	Deer	0.22	0.21	0.28
Extractable P	Worm	-0.11	0.21	0.6

Appendix II.15. Standard deviation of the random effect of site on all species, calculated using maximum likelihood. Soil nutrients are measured as concentrations (w/w) in the A horizon.

Species	Variable	Site	
		Intercept	Residual
Actaea	Biomass	0.07	0.08
	Mycorrhizae	4.07	9.56
	Fine root	0.03	0.08
	Total N	0.1	0.07
	Total P	0.04	0.15
	Extractable P	0.19	0.39
Aquilegia	Biomass	0.12	0.09
	Mycorrhizae	5.05	10.32
	Fine root	0.03	0.07
	Total N	0.1	0.08
	Total P	0.04	0.15
	Extractable P	0.18	0.39
Cornus	Biomass	0.13	0.03
	Mycorrhizae	3.44	9.31
	Fine root	0.03	0.1
	Total N	0.1	0.07
	Total P	0.04	0.15
	Extractable P	0.19	0.39
Prenanthes	Biomass	0	0.08
	Mycorrhizae	3.45	14.44
	Fine root	0	0.14
	Total N	0.1	0.07
	Total P	0.04	0.15
	Extractable P	0.19	0.39
Quercus	Biomass	0.28	0.09
	Mycorrhizae	1.04	9.8
	Fine root	0.03	0.08
	Total N	0.1	0.07
	Total P	0.05	0.14
	Extractable P	0.19	0.38

Appendix II.16. Correlated errors of fixed variables from seedling survival models. Soil nutrients are measured as concentrations (w/w) in the A horizon.

		Extractable P	Total P	Mycorrhizae	Total N
Actaea	Extractable P	-			
	Total P	NS	-		
	Mycorrhizae	NS	NS	-	
	Total N	NS	NS	NS	-
	Fine root	NS	0.16¥	NS	NS
Aqulegia	Extractable P	-			
	Total P	NS	-		
	Mycorrhizae	NS	NS	-	
	Total N	NS	NS	0.2¥	-
	Fine root	NS	0.19¥	NS	NS
Cornus	Extractable P	-			
	Total P	NS	-		
	Mycorrhizae	NS	NS	-	
	Total N	NS	NS	0.24*	-
	Fine root	NS	NS	NS	NS
Prenanthes	Extractable P	-			
	Total P	NS	-		
	Mycorrhizae	NS	NS	-	
	Total N	NS	NS	0.15¥	-
	Fine root	NS	NS	0.22*	0.37***
Quercus	Extractable P	-			
	Total P	NS	-		
	Mycorrhizae	NS	NS	-	
	Total N	NS	NS	NS	-
	Fine root	NS	NS	NS	NS

Appendix II.17. Individual model fits of biomass models using random and fixed effects (R^2_c) and fixed effects only (R^2_m). Soil nutrients are measured as pools in the top 20cm.

Species		R^2_m	R^2_c	n
Actaea	Biomass	0.22	0.22	28
	Mycorrhizae	0.00	0.11	35
	Fine roots	0.02	0.28	43
	Total N	0.06	0.64	40
	Total P	0.03	0.36	48
	Extractable P	0.23	0.74	48
Aquilegia	Biomass	0.36	0.36	17
	Mycorrhizae	0.09	0.20	27
	Fine roots	0.16	0.16	19
	Total N	0.07	0.62	30
	Total P	0.03	0.34	36
	Extractable P	0.23	0.73	36
Cornus	Biomass	0.05	0.05	37
	Mycorrhizae	0.06	0.26	29
	Fine roots	0.05	0.21	45
	Total N	0.06	0.64	40
	Total P	0.03	0.36	48
	Extractable P	0.23	0.74	48
Prenanthes	Biomass	0.10	0.19	19
	Mycorrhizae	0.05	0.05	19
	Fine roots	0.01	0.01	19
	Total N	0.06	0.64	40
	Total P	0.03	0.36	48
	Extractable P	0.23	0.74	48
Quercus	Biomass	0.15	0.15	29
	Mycorrhizae	0.03	0.12	38
	Fine roots	0.00	0.21	39
	Total N	0.06	0.65	50
	Total P	0.03	0.37	60
	Extractable P	0.23	0.75	60

Appendix II.18. Fixed model components for the effects of deer and earthworms on biomass of *Actaea pachypoda* (Fisher's C = 8.15; df = 10; p=0.61; AIC = 84.15; AICc = -409.85; k=38; n=33). Soil nutrients are measured as pools in the top 20cm. Significant paths are bolded.

Response	Predictor	Estimate	SE	p
Biomass	**Total N**	**-1.29**	**0.52**	**0.02**
Biomass	**Extractable P**	**1.12**	**0.51**	**0.04**
Biomass	Total P	-0.24	0.18	0.21
Biomass	Mycorrhizae	0.16	0.20	0.44
Biomass	Worm	-0.13	0.19	0.50
Biomass	Deer	-0.36	0.53	0.51
Biomass	Fine root	-0.07	0.16	0.66
Mycorrhizae	Worm	-0.05	0.18	0.77
Mycorrhizae	Deer	-0.07	0.41	0.86
Fine root	Worm	-0.14	0.14	0.30
Fine root	Deer	-0.10	0.30	0.74
Total N	**Worm**	**-0.42**	**0.10**	**0.00**
Total N	Deer	-0.38	0.27	0.17
Total P	Worm	-0.16	0.12	0.20
Total P	Deer	-0.19	0.26	0.48
Extractable P	**Worm**	**-0.58**	**0.10**	**0.00**
Extractable P	Deer	0.07	0.21	0.75

Appendix II.19. Fixed model components for the effects of deer and earthworms on biomass of *Aquilegia canadense* (Fisher's C = 8.31; df = 10; p=0.60; AIC = 80.31; AICc = -46.55; k=36; n=16). Soil nutrients are measured as pools in the top 20cm. Significant paths are bolded.

Response	Predictor	Estimate	SE	p
Biomass	Fine root	-0.67	0.36	0.10
Biomass	Worm	0.30	0.27	0.30
Biomass	Mycorrhizae	0.16	0.35	0.66
Biomass	Deer	-0.10	0.57	0.86
Mycorrhizae	Worm	0.24	0.18	0.19
Mycorrhizae	Deer	-0.25	0.39	0.53
Fine root	Worm	0.40	0.16	0.03
Fine root	Deer	0.52	0.33	0.14
Total N	**Worm**	**-0.42**	**0.12**	**0.00**
Total N	Deer	-0.39	0.32	0.23
Total P	Worm	-0.16	0.14	0.27
Total P	Deer	-0.18	0.31	0.55
Extractable P	**Worm**	**-0.58**	**0.11**	**0.00**
Extractable P	Deer	0.06	0.24	0.82

Appendix II.20. Fixed model components for the effects of deer and earthworms on biomass of *Cornus racemosa* (Fisher's C = 11.25; df = 12; p = 0.51; AIC = 81.25; AICc = 278.75; k = 35; n = 29). Soil nutrients are measured as pools in the top 20cm. Significant paths are bolded.

Response	Predictor	Estimate	SE	p
Biomass	Extractable P	0.51	0.50	0.31
Biomass	Fine root	-0.25	0.28	0.37
Biomass	Deer	-0.43	0.58	0.46
Biomass	Total N	-0.39	0.54	0.48
Biomass	Worm	0.10	0.23	0.68
Mycorrhizae	Worm	-0.21	0.15	0.17
Mycorrhizae	Deer	-0.19	0.34	0.58
Fine root	Worm	-0.15	0.11	0.20
Fine root	Deer	0.18	0.24	0.45
Total N	**Worm**	**-0.42**	**0.10**	**0.00**
Total N	Deer	-0.38	0.27	0.17
Total P	Worm	-0.16	0.12	0.20
Total P	Deer	-0.19	0.26	0.48
Extractable P	**Worm**	**-0.58**	**0.10**	**0.00**
Extractable P	Deer	0.07	0.21	0.75

Appendix II.21. Fixed model components for the effects of deer and earthworms on biomass of *Prenanthes alba* (Fisher's C = 11.72; df = 12; p = 0.47; AIC = 85.72; AICc = -54.88; k = 37; n = 18). Soil nutrients are measured as pools in the top 20cm. Significant paths are bolded.

Response	Predictor	Estimate	SE	p
Biomass	Worm	-0.22	0.28	0.45
Biomass	Total P	-0.27	0.38	0.50
Biomass	Deer	-0.35	0.51	0.51
Biomass	Extractable P	-0.09	0.22	0.69
Mycorrhizae	Deer	-0.55	0.48	0.27
Mycorrhizae	Worm	-0.14	0.25	0.58
Fine root	Deer	-0.24	0.49	0.63
Fine root	Worm	-0.01	0.26	0.97
Total N	**Worm**	**-0.42**	**0.10**	**0.00**
Total N	Deer	-0.38	0.27	0.17
Total P	Worm	-0.16	0.12	0.20
Total P	Deer	-0.19	0.26	0.48
Extractable P	**Worm**	**-0.58**	**0.10**	**0.00**
Extractable P	Deer	0.07	0.21	0.75

Appendix II.22. Fixed model components for the effects of deer and earthworms on biomass of *Quercus rubra* (Fisher's C = 5.43; df = 10; p = 0.86; AIC = 77.43; AICc = -1254.57; k = 36; n =35). Soil nutrients are measured as pools in the top 20cm. Significant paths are bolded.

Response	Predictor	Estimate	SE	p
Biomass	Total N	0.83	0.46	0.09
Biomass	Deer	0.66	0.48	0.19
Biomass	Extractable P	-0.51	0.46	0.28
Biomass	Mycorrhizae	0.16	0.25	0.53
Biomass	Worm	0.05	0.21	0.83
Mycorrhizae	Deer	0.34	0.35	0.34
Mycorrhizae	Worm	-0.04	0.15	0.77
Fine root	Deer	0.11	0.35	0.76
Fine root	Worm	-0.01	0.15	0.97
Total N	**Worm**	**-0.42**	**0.09**	**0.00**
Total N	Deer	-0.37	0.24	0.12
Total P	Worm	-0.15	0.11	0.16
Total P	Deer	-0.19	0.23	0.42
Extractable P	**Worm**	**-0.58**	**0.08**	**0.00**
Extractable P	Deer	0.07	0.18	0.69

Appendix II.23. Standard deviation of the random effect of site on all species on dry plant biomass, calculated using maximum likelihood. Soil nutrients are measured as pools in the top 20cm.

Species	Variable	Site	
		Intercept	Residual
Actaea	Biomass	0	0.42
	Mycorrhizae	3.84	10.95
	Fine root	0.05	0.08
	Total N	2.08	1.64
	Total P	0.28	0.39
	Extractable P	0.91	0.65
Aquilegia	Biomass	0.08	0.13
	Mycorrhizae	3.78	9.96
	Fine root	0	0.05
	Total N	2.08	1.64
	Total P	0.28	0.39
	Extractable P	0.91	0.65
Cornus	Biomass	0	0.06
	Mycorrhizae	3.93	7.76
	Fine root	0.03	0.08
	Total N	2.08	1.64
	Total P	0.28	0.39
	Extractable P	0.91	0.65
Prenanthes	Biomass	0.01	0.03
	Mycorrhizae	0	14.94
	Fine root	0	0.14
	Total N	2.08	1.64
	Total P	0.28	0.39
	Extractable P	0.91	0.65
Quercus	Biomass	0	0.26
	Mycorrhizae	2.94	9.36
	Fine root	0.04	0.08
	Total N	2.1	1.62
	Total P	0.28	0.39
	Extractable P	0.92	0.64

Appendix II.24. Correlated errors of fixed variables from dry plant biomass models. Soil nutrients are measured as pools in the top 20cm.

		Extractable P	Total P	Mycorrhizae	Total N
Actaea	Extractable P	-			
	Total P	NS	-		
	Mycorrhizae	NS	NS	-	
	Total N	0.91***	NS	0.28**	-
	Fine root	0.21*	NS	NS	0.31**
Aquelegia	Extractable P	-			
	Total P	NS	-		
	Mycorrhizae	0.4***	NS	-	
	Total N	0.91***	NS	0.4***	-
	Fine root	NS	NS	NS	NS
Cornus	Extractable P	-			
	Total P	NS	-		
	Mycorrhizae	0.28**	NS	-	
	Total N	0.91***	NS	0.34**	-
	Fine root	0.28**	NS	NS	0.36***
Prenanthes	Extractable P	-			
	Total P	NS	-		
	Mycorrhizae	NS	NS	-	
	Total N	0.91***	NS	NS	-
	Fine root	NS	NS	NS	NS
Quercus	Extractable P	-			
	Total P	NS	-		
	Mycorrhizae	NS	NS	-	
	Total N	0.91***	NS	NS	-
	Fine root	NS	NS	NS	0.32***

Appendix II.25. Individual model fits for survival models using random and fixed effects (R^2_c) and fixed effects only (R^2_m). Soil nutrients are measured as pools in the top 20cm.

Species		R^2_m	R^2_c	n
Actaea	Survival	0.40	0.97	28
	Mycorrhizae	0.00	0.11	35
	Fine root	0.02	0.28	43
	Total N	0.06	0.64	40
	Total P	0.03	0.36	48
	Extractable P	0.23	0.74	48
Aquilegia	Survival	0.18	0.41	18
	Mycorrhizae	0.09	0.20	27
	Fine roots	0.16	0.16	19
	Total N	0.07	0.62	30
	Total P	0.03	0.34	36
	Extractable P	0.23	0.73	36
Cornus	Survival	0.35	0.63	37
	Mycorrhizae	0.06	0.26	29
	Fine roots	0.05	0.21	45
	Total N	0.06	0.64	40
	Total P	0.03	0.36	48
	Extractable P	0.23	0.74	48
Prenanthes	Survival	0.81	0.81	48
	Mycorrhizae	0.05	0.05	19
	Fine roots	0.01	0.01	19
	Total N	0.06	0.64	40
	Total P	0.03	0.36	48
	Extractable P	0.23	0.74	48
Quercus	Survival	0.72	0.95	32
	Mycorrhizae	0.03	0.12	38
	Fine roots	0.00	0.21	39
	Total N	0.06	0.65	50
	Total P	0.03	0.37	60
	Extractable P	0.23	0.75	60

Appendix II.26. Fixed model components for the effects of deer and earthworms on survival of *Actaea pachypoda* (Fisher's C = 7.49; df = 10; p=0.68; AIC = 81.49; AICc = -480.91; k=37; n=33). Soil nutrients are measured as pools in the top 20cm. Significant paths are bolded.

Response	Predictor	Estimate	SE	p
Survival	**Earthworm**	**-0.54**	**0.07**	**0.00**
Survival	**Total P**	**-0.16**	**0.06**	**0.02**
Survival	**Fine roots**	**0.12**	**0.05**	**0.03**
Survival	**Deer**	**-0.39**	**0.18**	**0.04**
Survival	Extractable P	-0.36	0.17	0.05
Survival	Mycorrhizae	-0.05	0.06	0.36
Survival	Total N	0.02	0.17	0.89
Mycorrhizae	Earthworm	-0.05	0.18	0.77
Mycorrhizae	Deer	-0.07	0.41	0.86
Fine roots	Earthworm	-0.14	0.14	0.30
Fine roots	Deer	-0.10	0.30	0.74
Total N	**Earthworm**	**-0.42**	**0.10**	**0.00**
Total N	Deer	-0.38	0.27	0.17
Total P	Earthworm	-0.16	0.12	0.20
Total P	Deer	-0.19	0.26	0.48
Extractable P	**Earthworm**	**-0.58**	**0.10**	**0.00**
Extractable P	Deer	0.07	0.21	0.75

Appendix II.27. Fixed model components for the effects of deer and earthworms on survival of *Aquilegia canadense* (Fisher's C = 8.31; df = 10; p=0.6; AIC = 80.31; AICc = -52.89; k=36; n=17). Soil nutrients are measured as pools in the top 20cm. Significant paths are bolded.

Response	Predictor	Estimate	SE	p
Survival	Deer	0.51	0.22	0.05
Survival	Earthworm	0.24	0.11	0.05
Survival	Fine roots	-0.19	0.14	0.20
Survival	Mycorrhizae	-0.06	0.13	0.62
Mycorrhizae	Earthworm	0.24	0.18	0.19
Mycorrhizae	Deer	-0.25	0.39	0.53
Fine roots	**Earthworm**	**0.40**	**0.16**	**0.03**
Fine roots	Deer	0.52	0.33	0.14
Total N	**Earthworm**	**-0.42**	**0.12**	**0.00**
Total N	Deer	-0.39	0.32	0.23
Total P	Earthworm	-0.16	0.14	0.27
Total P	Deer	-0.18	0.31	0.55
Extractable P	**Earthworm**	**-0.58**	**0.11**	**0.00**
Extractable P	Deer	0.06	0.24	0.82

Appendix II.28. Fixed model components for the effects of deer and earthworms on survival of *Cornus racemosa* (Fisher's C = 13.79; df = 12; p = 0.31; AIC = 83.79; AICc = 276.21; k = 35; n = 29). Soil nutrients are measured as pools in the top 20cm. Significant paths are bolded.

Response	Predictor	Estimate	SE	p
Survival	**Earthworm**	**0.38**	**0.03**	**0.00**
Survival	**Extractable P**	**0.58**	**0.09**	**0.00**
Survival	**Deer**	**-0.33**	**0.09**	**0.00**
Survival	**Total N**	**-0.32**	**0.09**	**0.00**
Survival	Fine roots	0.02	0.04	0.57
Survival	Mycorrhizae	0.02	0.04	0.64
Mycorrhizae	Earthworm	-0.21	0.15	0.17
Mycorrhizae	Deer	-0.19	0.34	0.58
Fine roots	Earthworm	-0.15	0.11	0.20
Fine roots	Deer	0.18	0.24	0.45
Total N	**Earthworm**	**-0.42**	**0.10**	**0.00**
Total N	Deer	-0.38	0.27	0.17
Total P	Earthworm	-0.16	0.12	0.20
Total P	Deer	-0.19	0.26	0.48
Extractable P	**Earthworm**	**-0.58**	**0.10**	**0.00**
Extractable P	Deer	0.07	0.21	0.75

Appendix II.29. Fixed model components for the effects of deer and earthworms on survival of *Prenanthes alba* (Fisher's C = 10.82; df = 10; p = 0.37; AIC = 82.82; AICc = -57.4; k = 36; n = 18). Soil nutrients are measured as pools in the top 20cm. Significant paths are bolded.

Response	Predictor	Estimate	SE	p
Survival	**Total P**	**-0.81**	**0.08**	**0.00**
Survival	**Extractable P**	**0.46**	**0.08**	**0.00**
Survival	**Deer**	**0.46**	**0.15**	**0.00**
Survival	**Earthworm**	**-0.21**	**0.09**	**0.02**
Mycorrhizae	Deer	-0.55	0.48	0.27
Mycorrhizae	Earthworm	-0.14	0.25	0.58
Fine roots	Deer	-0.24	0.49	0.63
Fine roots	Earthworm	-0.01	0.26	0.97
Total N	**Earthworm**	**-0.42**	**0.10**	**0.00**
Total N	Deer	-0.38	0.27	0.17
Total P	Earthworm	-0.16	0.12	0.20
Total P	Deer	-0.19	0.26	0.48
Extractable P	**Earthworm**	**-0.58**	**0.10**	**0.00**
Extractable P	Deer	0.07	0.21	0.75

Appendix II.30. Fixed model components for the effects of deer and earthworms on survival of *Quercus rubra* (Fisher's C = 5.43; df = 10; p = 0.86; AIC = 77.43; AICc = -2386.57; k = 36; n =36). Soil nutrients are measured as pools in the top 20cm. Significant paths are bolded.

Response	Predictor	Estimate	SE	p
Survival	**Total N**	**-1.20**	**0.18**	**0.00**
Survival	**Extractable P**	**0.91**	**0.16**	**0.00**
Survival	**Earthworm**	**-0.22**	**0.05**	**0.00**
Survival	Deer	-0.36	0.18	0.06
Survival	Mycorrhizae	0.10	0.07	0.13
Survival	Fine roots	0.04	0.05	0.38
Mycorrhizae	Deer	0.34	0.35	0.34
Mycorrhizae	Earthworm	-0.04	0.15	0.77
Fine roots	Deer	0.11	0.35	0.76
Fine roots	Earthworm	-0.01	0.15	0.97
Total N	**Earthworm**	**-0.42**	**0.09**	**0.00**
Total N	Deer	-0.37	0.24	0.12
Total P	Earthworm	-0.15	0.11	0.16
Total P	Deer	-0.19	0.23	0.42
Extractable P	**Earthworm**	**-0.58**	**0.08**	**0.00**
Extractable P	Deer	0.07	0.18	0.69

Appendix II.31. Standard deviation of the random effect of site on all species, calculated using maximum likelihood. Soil nutrients are measured as pools in the top 20cm.

Species	Variable	Site	
		Intercept	Residual
Actaea	Survival	0.12	0.03
	Mycorrhizae	3.84	10.95
	Fine root	0.05	0.08
	Total N	2.08	1.64
	Total P	0.28	0.39
	Extractable P	0.91	0.65
Aquilegia	Survival	0.05	0.13
	Mycorrhizae	3.78	9.96
	Fine root	2.62	0.05
	Total N	2.04	1.69
	Total P	0.27	0.4
	Extractable P	0.9	0.67
Cornus	Survival	0.08	0.1
	Mycorrhizae	3.93	7.76
	Fine root	0.033	0.075
	Total N	2.08	1.64
	Total P	0.28	0.39
	Extractable P	0.91	0.65
Prenanthes	Survival	2.78	0.15
	Mycorrhizae	0	14.94
	Fine root	0	0.14
	Total N	2.08	1.64
	Total P	0.28	0.39
	Extractable P	0.91	0.65
Quercus	Survival	0.11	0.05
	Mycorrhizae	2.94	9.36
	Fine root	0.04	0.08
	Total N	2.1	1.62
	Total P	0.28	0.39
	Extractable P	0.92	0.64

Appendix II.32. Correlated errors of fixed variables from seedling survival models. Soil nutrients are measured as pools in the top 20cm.

		Extractable P	Total P	Mycorrhizae	Total N
Actaea	Extractable P	-			
	Total P	NS	-		
	Mycorrhizae	NS	NS	-	
	Total N	0.91***	NS	0.28**	-
	Fine root	0.21*	NS	NS	0.31**
Aquilegia	Extractable P	-			
	Total P	NS	-		
	Mycorrhizae	0.4***	NS	-	
	Total N	0.91***	NS	0.4***	-
	Fine root	NS	NS	NS	NS
Cornus	Extractable P	-			
	Total P	NS	-		
	Mycorrhizae	0.28**	NS	-	
	Total N	0.91***	NS	0.34**	-
	Fine root	0.28**	NS	NS	0.36***
Prenanthes	Extractable P	-			
	Total P	NS	-		
	Mycorrhizae	NS	NS	-	
	Total N	0.91***	NS	NS	-
	Fine root	NS	NS	NS	NS
Quercus	Extractable P	-			
	Total P	NS	-		
	Mycorrhizae	NS	NS	-	
	Total N	0.91***	NS	NS	-
	Fine root	0.16*	NS	NS	0.32***

Appendix II.33. Individual model fits for DSE models using random and fixed effects (R^2_c) and fixed effects only (R^2_m). Soil nutrients are measured as concentrations (w/w) in the A horizon.

	R2m	R2c	n
DSE	0.05	0.65	165
Mycorrhizae	0.01	0.35	267
Total P	0.6	0.65	368
Extractable P	0.01	0.25	368
Total N	0.2	0.74	276

Appendix II.34. Fixed model components for the effects of deer and earthworms on root colonization by dark septate endophytes (DSE) (Fisher's C = 3.24; df = 2; p = 0.20; AIC = 67.24; AICc = 83.24; k = 32; n = 165). Soil nutrients are measured as concentrations (w/w) in the A horizon.

Response	predictor	estimate	SE	p
DSE	**Total N**	**0.69**	**0.26**	**0.007**
DSE	Deer	0.3	0.31	0.34
DSE	Extractable P	0.26	0.28	0.35
DSE	Mycorrhizae	-0.24	0.27	0.37
DSE	Worm	-0.2	0.3	0.5
DSE	Total P	0.11	0.4	0.8
Mycorrhizae	Worm	-0.01	0.06	0.85
Mycorrhizae	Deer	-0.01	0.06	0.9
Total P	**Deer**	**-0.63**	**0.03**	**0**
Total P	**Worm**	**-0.46**	**0.03**	**0**
Extractable P	Deer	**0.11**	**0.05**	**0.017**
Extractable P	Worm	-0.054	0.05	0.25
Total N	Deer	**-0.56**	**0.05**	**0**
Total N	**Worm**	**0.05**	**0.04**	**0.24**

Appendix II.35. DSE Random effects (variance and standard deviation) of species and site for biomass models. Soil nutrients are measured as concentrations (w/w) in the A horizon.

	Species		Site		Residual	
	Var	SD	Var	SD	Var	SD
DSE	5.7	2.39	0	0		
Mycorrhizae	57.92	7.61	2.6	1.61	113.74	10.67
Total P	0	0	0.003	0.055	0.02	0.14
Extractable P	0	0	0.04	0.21	0.14	0.37
Total N	0	0	0.01	0.1	0.005	0.071

Appendix II.36. Correlated errors of fixed predictors of DSE colonization. Soil nutrients are measured as concentrations (w/w) in the A horizon.

	Extractable	Total P	Mycorrhizae	Total N
Extractable P	-			
Total P	NS	-		
Mycorrhizae	NS	NS	-	
Total N	NS	NS	0.2***	-

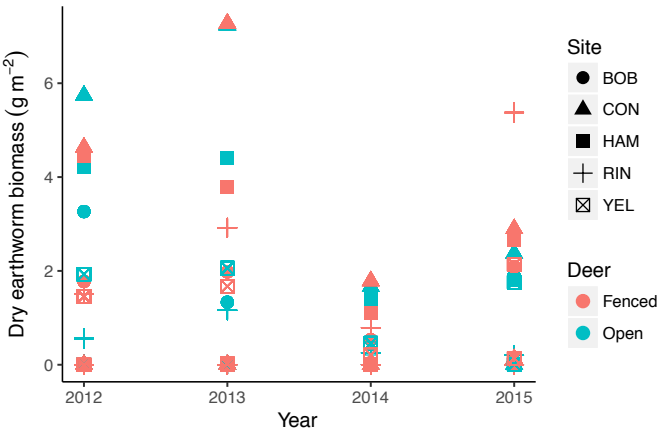
APPENDIX III

Appendix III.1. Planting and trait details for transplants in forested plots in central NY from 2012 – 2017. Trait data for SLA and Foliar N were collected from the TRY database. Palatability to deer and shade tolerance were compiled from Brundrett and Kendrick (1988), Jull (2001), Heckel et al. (2010), Dávalos et al. (2014), Scala et al. (2014), Bachand et al. (2015), Heberling et al. (2017) and personal observation.

Species	Life form	Planting date	# planted	# surviving at experiment end	# flowering at experiment end	Palatability to deer	Insect attack α	Shade tolerance	Mean SLA (cm2/g)	# of SLA measurements	Datasets	Mean Foliar N (%)	# of Foliar N measurements	Datasets
Actaea rubra	Perrenial herb	Spring 2012	400	31	1	Low	Moderate	Tolerant	406.14	22	Akira Mori, Brandon Schamp, Evan Weiher,	2.94	4	Peter Reich, Akira Mori
Adiantum pedatum	Fern	Spring 2013	400	35	NA	Low	Moderate	Tolerant	1027.33	7	Evan Weiher, Brandon Schamp, Sandy Harrison,	1.88	1	Vincent Marie
Allium canadense	Perrenial herb	Spring 2012	400	40	0	Moderate	Low	Moderate	202.68	8	Brandon Schamp, Ian Wright, Bill Shippley	2.59	0	
Agrimonia gryposepala β	Perrenial herb	Spring 2012	400	8	1	High	Moderate	Intolerant	454.00	0		2.20	1	Joseph Craine
Arisaema triphyllum	Perrenial herb	Spring 2013	200	95	4	Low	Moderate	Moderate	355.92	36	Evan Weiher, Brandon Schamp, Ian Wright, Jens,	3.67	8	Peter Reich, Ian Wright, Jens Kattge, Peter Adler
Brachyelytrum erectum	Grass	Fall 2013	200	42	9	High	High	Moderate	508.95	3	Brandon Schamp	3.47	1	Peter Reich
Carex radiata	Sedge	Spring 2012	400	61	30	Low	Low	Moderate	NA	NA	NA	NA	NA	NA
Caulophyllum thalictroides	Perrenial herb	Spring 2012	400	134	0	Low	Low	Tolerant	232.26	12	Evan Weiher, Brandon Schamp, Ian Wright, Jens	4.20	5	Peter Reich, Ian Wright, Jens Kattge, Vincent Marie
Dryopteris sp	Fern	Spring 2013	400	282	NA	Low	Moderate	Moderate	241.55	4	Brandon Schamp, Vincent	2.57	2	Isabelle Aubin, Vincent Marie
Geranium maculatum β	Perrenial herb	Spring 2012	300	13	0	High	Moderate	Intolerant	94.58	15	Evan Weiher	2.08	3	Peter Reich, Robert Jackson
Geum canadense β	Perrenial herb	Spring 2012	400	3	1	High	Moderate	Intolerant	291.20	52	Evan Weiher, Brandon Schamp, Joseph Craine	1.32	2	Peter Reich, Joseph Craine
Maianthemum canadense	Perrenial herb	Fall 2013	200	58	2	High	Moderate	Tolerant	2373.32	116	Peter Reich, Brandon Schamp, Evan Weiher, Ian	2.05	81	Bill Shipley, Peter Reich, Isabelle Aubin
Polygonatum biflorum	Perrenial herb	Spring 2012	400	142	5	High	Moderate	Tolerant	254.86	3	Brandon Schamp	2.21	2	Peter Reich
Polygonum virginiana β	Perrenial herb	Spring 2012	400	13	0	High	High	Intolerant	454.00	0		2.09	1	Joseph Craine
Polystichum acrostichoides	Fern	Spring 2013	400	174	NA	Low	Low	Moderate	182.48	1	Vincent Marie	2.24	7	Robert Jackson, Vincent Marie
Quercus rubra	Tree	Fall 2013	400	56	0	High	High	Tolerant	99.37	101	Peter Reich, Brandon Schamp, Evan Weiher, Michael Kleyer, Belinda Medlyn, Ian Wright, Jens	2.11	154	Joseph Craine, Peter Reich, Belinda Medlyn, Peter Reich, Brian Enquist, Ian Wright, Johannes Cornelissen, Jens
Sanguinaria canadensis	Perrenial herb	Spring 2013	400	49	1	Low	Low	Tolerant	250.76	19	Brandon Schamp, Evan Weiher, Ian Wright, Jens	3.58	8	Peter Reich, Ian Wright, Jens Kattge, Vincent Marie
Thalictrum dioicum	Perrenial herb	Fall 2013	300	69	3	High	High	Moderate	413.88	29	Evan Weiher, Brandon Schamp	1.68	4	Peter Reich
Tiarella cordifolia	Perrenial herb	Spring 2013	400	80	14	Moderate	High	Tolerant	454.00	0		1.49	2	Peter Reich
Trillium erectum	Perrenial herb	Spring 2012	400	87	2	High	High	Tolerant	454.00	0		2.67	1	Peter Reich

α As observed in this experiment

β Removed from height and width analysis due to low survival



Appendix III.2. Dry earthworm biomass (g m^{-2}) measured with ustard extraction from 2012 – 2015. Color represents fencing (open in blue, fenced in red) and shape represents individual sites (n=5). Points are generated from the mean of ten 0.5 x 0.5 m quadrats.

Appendix III.3. Model output for GLMMs with Binomial distribution for transplant survival (n = 200 – 400 per species) in forested plots in central NY from 2012 - 2017. Models include random effects of site and plot within site reported in Appendix III.6.

Species	Parameter	Estimate	SE	Z	p
Actaea	**Intercept**	**2.64**	**0.36**	**7.42**	**<0.001**
	Initial size	**0.16**	**0.04**	**3.90**	**<0.001**
	Deer	0.07	0.19	0.35	0.729
	Earthworm	-0.30	0.29	-1.03	0.305
	Year	**-1.14**	**0.06**	**-18.08**	**<0.001**
	Deer x Earthworm	-0.32	0.22	-1.47	0.142
	Year x Earthworm	**0.18**	**0.08**	**2.24**	**0.025**
Adiantum	**Intercept**	**2.68**	**0.39**	**6.84**	**<0.001**
	Initial size	**0.14**	**0.02**	**5.68**	**<0.001**
	Deer	-0.20	0.21	-0.97	0.332
	Earthworm	**-2.06**	**0.44**	**-4.71**	**<0.001**
	Year	**-1.54**	**0.13**	**-12.01**	**<0.001**
	Deer x Earthworm	**0.58**	**0.26**	**2.29**	**0.022**
	Year x Earthworm	**0.96**	**0.14**	**6.91**	**<0.001**
Allium	Intercept	0.47	0.43	1.09	0.277
	Initial size	**0.10**	**0.02**	**6.48**	**<0.001**
	Deer	0.07	0.16	0.42	0.675
	Earthworm	-0.24	0.34	-0.70	0.484
	Year	**-0.79**	**0.07**	**-11.82**	**<0.001**
	Deer x Earthworm	**-0.67**	**0.22**	**-3.04**	**0.002**
	Year x Earthworm	**0.31**	**0.08**	**3.63**	**<0.001**
Agrimonia	**Intercept**	**0.75**	**0.38**	**2.00**	**0.045**
	Initial size	**0.28**	**0.03**	**9.21**	**<0.001**
	Deer	0.06	0.28	0.23	0.820
	Earthworm	-0.57	0.34	-1.70	0.090
	Year	**-1.53**	**0.11**	**-13.48**	**<0.001**
	Deer x Earthworm	-0.18	0.28	-0.64	0.525
	Year x Earthworm	**0.58**	**0.13**	**4.52**	**<0.001**
Arisaema	**Intercept**	**6.86**	**0.77**	**8.80**	**<0.001**
	Initial size	-0.07	0.06	-1.13	0.294
	Year	**-1.03**	**0.11**	**-9.30**	**<0.001**
	Deer	-0.22	0.37	-0.61	0.540
	Earthworm	0.29	0.32	0.91	0.362
	Deer x Earthworm	-0.80	0.42	-1.88	0.060
Brachyelytrum	Intercept	0.47	0.44	1.08	0.281
	Initial size	**0.08**	**0.02**	**3.13**	**0.002**
	Deer	-0.41	0.53	-0.77	0.439
	Earthworm	**-1.90**	**0.53**	**-3.60**	**<0.001**
	Year	**-0.47**	**0.10**	**-4.76**	**<0.001**
	Deer x Earthworm	**1.81**	**0.73**	**2.48**	**0.013**
	Year x Deer	0.19	0.13	1.43	0.153
	Year x Earthworm	**0.64**	**0.13**	**4.97**	**<0.001**
	Deer x Year x Earthworm	**-0.52**	**0.18**	**-2.96**	**0.003**
Carex	**Intercept**	**2.42**	**0.31**	**7.89**	**<0.001**

	Initial size	**0.07**	**0.02**	**2.90**	**0.004**
	Deer	0.13	0.20	0.64	0.522
	Earthworm	-0.15	0.28	-0.55	0.582
	Year	**-1.00**	**0.06**	**-17.76**	**<0.001**
	Deer x Earthworm	0.06	0.21	0.28	0.780
	Year x Earthworm	**0.36**	**0.07**	**4.92**	**<0.001**
Caulophyllum	**Intercept**	**1.24**	**0.36**	**3.46**	**<0.001**
	Initial size	**0.19**	**0.03**	**7.29**	**<0.001**
	Year	**-0.75**	**0.05**	**-15.06**	**<0.001**
	Deer	-0.24	0.28	-0.87	0.384
	Earthworm	0.34	0.18	1.91	0.056
	Deer x Earthworm	0.07	0.28	0.29	0.790
Dryopteris	**Intercept**	**5.72**	**0.73**	**7.80**	**<0.001**
	Initial size	**0.02**	**0.01**	**3.81**	**<0.001**
	Species [marginalis]	**-0.58**	**0.17**	**-3.36**	**0.001**
	Deer	0.97	1.08	0.90	0.367
	Earthworm	**7.93**	**2.90**	**2.73**	**0.006**
	Year	**-0.84**	**0.14**	**-6.08**	**<0.001**
	Deer x Earthworm	**-7.24**	**3.16**	**-2.29**	**0.022**
	Year x Deer	-0.21	0.20	-1.05	0.293
	Year x Earthworm	**-1.23**	**0.50**	**-2.44**	**0.015**
	Deer x Year x Earthworm	**1.16**	**0.56**	**2.09**	**0.037**
Geum	**Intercept**	**3.31**	**0.49**	**6.70**	**<0.001**
	Initial size	**0.22**	**0.03**	**6.49**	**<0.001**
	Deer	-0.41	0.40	-1.02	0.309
	Earthworm	**-1.55**	**0.43**	**-3.62**	**<0.001**
	Year	**-3.18**	**0.25**	**-12.84**	**<0.001**
	Deer x Earthworm	**-0.83**	**0.33**	**-2.51**	**0.012**
	Year x Earthworm	**1.64**	**0.24**	**6.98**	**<0.001**
	Year x Deer	**0.45**	**0.16**	**2.77**	**0.006**
Geranium	**Intercept**	**2.32**	**0.75**	**3.09**	**0.002**
	Initial size	**0.23**	**0.04**	**6.68**	**<0.001**
	Deer	-0.15	0.31	-0.49	0.622
	Earthworm	-1.24	0.92	-1.35	0.176
	Year	**-1.29**	**0.19**	**-6.67**	**<0.001**
	Deer x Earthworm	-0.34	0.41	-0.83	0.408
	Year x Earthworm	**0.54**	**0.23**	**2.33**	**0.020**
Maianthemum	**Intercept**	**-0.53**	**0.20**	**-2.62**	**0.009**
	Initial size	**0.12**	**0.02**	**5.22**	**<0.001**
	Deer	-0.06	0.19	-0.32	0.753
	Earthworm	**-0.35**	**0.19**	**-1.84**	**0.050**
	Deer x Earthworm	-0.31	0.27	-1.16	0.245
Polygonatum	**Intercept**	**5.38**	**0.47**	**11.39**	**<0.001**
	Initial size	**0.10**	**0.02**	**5.77**	**<0.001**
	Deer	-0.24	0.28	-0.87	0.382
	Earthworm	**-3.16**	**0.42**	**-7.54**	**<0.001**
	Year	**-1.05**	**0.07**	**-14.38**	**<0.001**
	Deer x Earthworm	**0.63**	**0.23**	**2.78**	**0.005**

	Year x Earthworm	**0.42**	**0.09**	**4.88**	**<0.001**
Polygonum	**Intercept**	**5.53**	**0.48**	**11.51**	**<0.001**
	Initial size	0.00	0.00	-0.13	0.897
	Deer	-0.31	0.30	-1.00	0.316
	Earthworm	**-2.17**	**0.52**	**-4.17**	**<0.001**
	Year	**-2.07**	**0.15**	**-14.04**	**<0.001**
	Deer x Earthworm	-0.03	0.27	-0.11	0.912
	Year x Earthworm	**1.01**	**0.16**	**6.22**	**<0.001**
Polystichum	**Intercept**	**3.27**	**0.49**	**6.73**	**<0.001**
	Initial size	**0.03**	**0.00**	**8.12**	**<0.001**
	Deer	0.64	0.64	1.01	0.315
	Earthworm	**3.74**	**0.91**	**4.11**	**<0.001**
	Year	**-0.80**	**0.09**	**-9.02**	**<0.001**
	Deer x Earthworm	**-3.32**	**1.15**	**-2.88**	**0.004**
	Year x Deer	-0.07	0.13	-0.54	0.589
	Year x Earthworm	**-0.45**	**0.18**	**-2.51**	**0.012**
	Deer x Year x Earthworm	**0.56**	**0.23**	**2.43**	**0.015**
Quercus	**Intercept**	**1.73**	**0.47**	**3.68**	**<0.001**
	Initial size	**0.04**	**0.02**	**2.16**	**0.031**
	Deer	-0.41	0.44	-0.93	0.352
	Earthworm	**1.04**	**0.34**	**3.02**	**0.003**
	Year	**-0.74**	**0.08**	**-9.70**	**<0.001**
	Deer x Earthworm	**-0.56**	**0.22**	**-2.60**	**0.009**
	Year x Earthworm	**-0.17**	**0.08**	**-2.11**	**0.035**
	Year x Deer	**0.23**	**0.09**	**2.66**	**0.008**
Sanguinaria	Intercept	-0.33	0.32	-1.04	0.299
	Initial size	**0.23**	**0.05**	**4.69**	**<0.001**
	Deer	0.38	0.32	1.21	0.225
	Earthworm	**-0.66**	**0.33**	**-2.03**	**0.042**
	Year	**-0.36**	**0.07**	**-5.04**	**<0.001**
	Deer x Earthworm	**0.60**	**0.27**	**2.27**	**0.023**
	Year x Earthworm	**0.29**	**0.08**	**3.52**	**<0.001**
	Year x Deer	**-0.19**	**0.08**	**-2.35**	**0.019**
Thalictrum	**Intercept**	**3.47**	**0.36**	**9.64**	**<0.001**
	Initial size	0.02	0.02	0.90	0.368
	Deer	-0.06	0.18	-0.36	0.721
	Earthworm	-0.44	0.42	-1.06	0.288
	Year	**-1.08**	**0.08**	**-13.77**	**<0.001**
	Deer x Earthworm	**-0.57**	**0.24**	**-2.39**	**0.017**
	Year x Earthworm	**0.45**	**0.10**	**4.62**	**<0.001**
Tiarella	Intercept	3.69	0.17	0.99	0.321
	Initial size	-0.01	0.01	-0.78	0.433
	Year	**-0.86**	**0.05**	**-17.80**	**<0.001**
	Deer	-0.40	0.32	-1.27	0.205
	Earthworm	0.10	0.17	0.57	0.572
	Deer x Earthworm	**-0.62**	**0.24**	**-2.59**	**0.010**
Trillium	**Intercept**	**2.95**	**0.32**	**9.32**	**<0.001**
	Initial size	-0.01	0.02	-0.26	0.794

Deer	-0.09	0.18	-0.50	0.618
Earthworm	**-1.41**	**0.33**	**-4.32**	**<0.001**
Year	**-0.70**	**0.05**	**-12.86**	**<0.001**
Deer x Earthworm	-0.15	0.20	-0.74	0.460
Year x Earthworm	**0.26**	**0.07**	**3.46**	**0.001**

Appendix III.4. Model output for LMMs with Gaussian distribution for transplant height (n = 31 - 282 per species at end of experiment) in forested plots in central NY from 2012 - 2017. Models include random effects of site and plot within site reported in Appendix III.6.

Species	Parameter	Estimate	SE	Z	p
Actaea	**Intercept**	**1.91**	**0.35**	**5.54**	**<0.001**
	Initial size	**0.34**	**0.05**	**6.97**	**<0.001**
	Deer	-0.67	0.37	-1.81	0.070
	Earthworm	-0.36	0.35	-1.02	0.308
	Year	**0.48**	**0.11**	**4.34**	**<0.001**
	Deer x Earthworm	**1.18**	**0.51**	**2.32**	**0.020**
	Year x Deer	**0.45**	**0.16**	**2.79**	**0.005**
	Deer x Year x Earthworm	**-0.80**	**0.22**	**-3.70**	**<0.001**
Adiantum	**Intercept**	**8.34**	**1.36**	**6.14**	**<0.001**
	Initial size	0.04	0.11	0.39	0.693
	Deer	**3.19**	**1.60**	**2.00**	**0.046**
	Earthworm	-0.89	0.80	-1.11	0.267
	Year	**1.51**	**0.35**	**4.30**	**<0.001**
	Deer x Earthworm	1.47	1.14	1.28	0.199
	Year x Deer	**-1.81**	**0.49**	**-3.68**	**<0.001**
Allium	**Intercept**	**3.54**	**0.67**	**5.30**	**<0.001**
	Initial size	0.04	0.04	1.15	0.252
	Deer	**1.60**	**0.71**	**2.24**	**0.025**
	Earthworm	-0.46	0.39	-1.18	0.237
	Year	**1.73**	**0.14**	**12.77**	**<0.001**
	Deer x Earthworm	0.00	0.56	0.00	0.998
	Year x Deer	**-0.64**	**0.20**	**-3.29**	**0.001**
Arisaema	**Intercept**	**9.06**	**0.66**	**13.66**	**<0.001**
	Initial size	**0.30**	**0.09**	**3.38**	**0.001**
	Deer	-0.08	0.40	-0.21	0.835
	Earthworm	0.37	0.40	0.92	0.359
	Deer x Earthworm	-0.07	0.58	-0.12	0.902
Brachyelytrum	Intercept	1.14	2.03	0.56	0.573
	Initial size	**0.68**	**0.11**	**5.92**	**<0.001**
	Deer	4.60	2.45	1.88	0.060
	Earthworm	**4.17**	**0.98**	**4.26**	**<0.001**
	Year	0.48	0.39	1.25	0.213
	Deer x Earthworm	1.06	1.28	0.83	0.406
	Year x Deer	**-1.29**	**0.53**	**-2.41**	**0.016**
Caulophllum	**Intercept**	**7.35**	**1.77**	**4.14**	**<0.001**
	Initial size	0.08	0.08	0.98	0.329
	Deer	**-4.98**	**2.39**	**-2.09**	**0.037**
	Earthworm	-2.17	2.14	-1.02	0.310
	Year	0.38	0.38	1.00	0.317
	Deer x Earthworm	**7.28**	**3.18**	**2.29**	**0.022**
	Year x Deer	**1.36**	**0.59**	**2.30**	**0.022**
	Year x Earthworm	0.67	0.51	1.30	0.193
	Deer x Year x Earthworm	**-2.00**	**0.77**	**-2.60**	**0.009**

Dryopteris	**Intercept**	**14.30**	**1.81**	**7.88**	**<0.001**
	Initial size	0.00	0.00	-1.79	0.073
	Species [marginalis]	**2.95**	**0.59**	**5.01**	**<0.001**
	Deer	3.14	2.28	1.37	0.169
	Earthworm	**-4.23**	**1.80**	**-2.34**	**0.019**
	Year	**2.77**	**0.35**	**7.86**	**<0.001**
	Deer x Earthworm	1.75	1.14	1.53	0.127
	Year x Earthworm	**1.31**	**0.41**	**3.23**	**0.001**
	Year x Deer	**-1.59**	**0.41**	**-3.92**	**<0.001**
Maianthemum	Intercept	0.03	0.59	0.05	0.963
	Initial size	**0.74**	**0.04**	**16.61**	**<0.001**
	Deer	-0.43	0.49	-0.87	0.382
	Earthworm	-0.93	0.73	-1.28	0.199
	Year	**0.93**	**0.12**	**7.55**	**<0.001**
	Deer x Earthworm	-0.36	0.51	-0.70	0.485
	Year x Earthworm	**0.51**	**0.18**	**2.75**	**0.006**
Polygonatum	**Intercept**	**1.73**	**0.51**	**3.37**	**0.001**
	Initial size	**0.44**	**0.03**	**14.68**	**<0.001**
	Deer	0.75	0.58	1.29	0.198
	Earthworm	**0.64**	**0.29**	**2.21**	**0.027**
	Year	**0.73**	**0.10**	**7.27**	**<0.001**
	Deer x Earthworm	-0.08	0.41	-0.19	0.848
	Year x Deer	**-0.30**	**0.14**	**-2.10**	**0.036**
Polystichum	**Intercept**	**16.35**	**2.01**	**8.15**	**<0.001**
	Initial size	**0.36**	**0.01**	**23.94**	**<0.001**
	Deer	3.04	2.36	1.29	0.197
	Earthworm	-2.53	1.96	-1.29	0.197
	Year	**-1.17**	**0.42**	**-2.76**	**0.006**
	Deer x Earthworm	-1.36	1.34	-1.01	0.311
	Year x Earthworm	**2.04**	**0.46**	**4.42**	**<0.001**
	Year x Deer	**-1.21**	**0.46**	**-2.65**	**0.008**
Quercus	**Intercept**	**-1.62**	**0.67**	**-2.41**	**0.016**
	Initial size	**0.77**	**0.04**	**19.13**	**<0.001**
	Deer	**1.85**	**0.66**	**2.81**	**0.005**
	Earthworm	**0.82**	**0.34**	**2.37**	**0.018**
	Year	**1.96**	**0.14**	**14.21**	**<0.001**
	Deer x Earthworm	-0.80	0.49	-1.63	0.103
	Year x Deer	**-0.73**	**0.19**	**-3.91**	**<0.001**
Thalictrum	Intercept	1.43	1.22	1.18	0.239
	Initial size	**0.32**	**0.06**	**5.30**	**<0.001**
	Deer	**2.34**	**1.07**	**2.19**	**0.028**
	Earthworm	**-3.94**	**1.19**	**-3.30**	**0.001**
	Year	**1.24**	**0.33**	**3.71**	**<0.001**
	Deer x Earthworm	0.12	0.83	0.14	0.887
	Year x Earthworm	**1.66**	**0.35**	**4.71**	**<0.001**
	Year x Deer	**-1.09**	**0.32**	**-3.42**	**0.001**

Appendix III.5. Model output for LMMs with Gaussian distribution for transplant width (n = 31 - 174 per species at end of experiment) in forested plots in central NY from 2012 - 2017. Models include random effects of site and plot within site reported in Appendix III.6.

Species	Parameter	Estimate	SE	Z	p
Actaea	**Intercept**	**-1.98**	**0.84**	**-2.36**	**0.018**
	Initial size	**0.49**	**0.10**	**4.73**	**<0.001**
	Deer	-1.82	1.18	-1.54	0.124
	Earthworm	-1.30	1.01	-1.29	0.196
	Year	**1.61**	**0.27**	**6.06**	**<0.001**
	Deer x Earthworm	2.30	1.48	1.55	0.121
	Year x Deer	**0.97**	**0.40**	**2.44**	**0.015**
	Year x Earthworm	**0.71**	**0.35**	**2.03**	**0.043**
	Deer x Year x Earthworm	**-1.20**	**0.52**	**-2.31**	**0.021**
Adiantum	**Intercept**	**4.40**	**1.79**	**2.46**	**0.014**
	Initial size	**0.42**	**0.13**	**3.35**	**0.001**
	Deer	0.42	1.24	0.34	0.731
	Earthworms	0.45	1.00	0.45	0.652
	Year	**1.04**	**0.41**	**2.56**	**0.010**
	Deer x Earthworms	0.33	1.42	0.23	0.814
Allium	**Intercept**	**-0.46**	**0.12**	**-3.80**	**<0.001**
	Initial size	**0.02**	**0.01**	**4.12**	**<0.001**
	Deer	**0.32**	**0.12**	**2.79**	**0.005**
	Earthworm	**0.22**	**0.11**	**2.02**	**0.044**
	Year	**0.38**	**0.03**	**12.03**	**<0.001**
	Deer x Earthworm	-0.04	0.09	-0.51	0.608
	Year x Earthworm	**-0.10**	**0.03**	**-2.96**	**0.003**
	Year x Deer	**-0.16**	**0.03**	**-4.89**	**<0.001**
Caulophllum	**Intercept**	**2.23**	**0.92**	**2.42**	**0.016**
	Initial size	**0.16**	**0.06**	**2.69**	**0.007**
	Deer	-0.81	0.55	-1.47	0.141
	Earthworm	-0.45	0.95	-0.48	0.633
	Year	**0.99**	**0.21**	**4.74**	**<0.001**
	Deer x Earthworm	0.40	0.70	0.56	0.574
Polygonatum	**Intercept**	**1.17**	**0.11**	**10.31**	**<0.001**
	Initial size	0.01	0.01	0.68	0.494
	Deer	0.00	0.11	0.01	0.994
	Earthworm	0.17	0.12	1.43	0.153
	Deer x Earthworm	0.14	0.17	0.87	0.385
Quercus	**Intercept**	**7.18**	**0.90**	**7.94**	**<0.001**
	Initial size	0.04	0.04	1.04	0.298
	Deer	1.87	1.05	1.79	0.074
	Earthworm	**0.93**	**0.35**	**2.62**	**0.009**
	Year	-0.07	0.17	-0.41	0.681
	Deer x Earthworm	-0.97	0.51	-1.88	0.059
	Year x Deer	**-0.47**	**0.23**	**-2.02**	**0.043**
Sanguinaria	Intercept	-0.07	0.48	-0.14	0.889
	Initial size	**0.73**	**0.06**	**11.96**	**<0.001**
	Deer	**1.93**	**0.48**	**4.05**	**<0.001**

	Earthworm	**-1.59**	**0.52**	**-3.09**	**0.002**
	Year	**0.43**	**0.15**	**2.80**	**0.005**
	Deer x Earthworm	-0.06	0.34	-0.18	0.856
	Year x Earthworm	**0.83**	**0.17**	**4.92**	**<0.001**
	Year x Deer	**-0.89**	**0.15**	**-5.83**	**<0.001**
Tiarella	**Intercept**	**-1.67**	**0.37**	**-4.50**	**<0.001**
	Initial size	**0.98**	**0.01**	**65.67**	**<0.001**
	Deer	0.56	0.46	1.22	0.224
	Earthworm	-0.54	0.44	-1.22	0.222
	Year	**1.01**	**0.10**	**10.00**	**<0.001**
	Deer x Earthworm	-0.31	0.31	-0.99	0.320
	Year x Earthworm	**0.40**	**0.12**	**3.37**	**0.001**
	Year x Deer	**-0.30**	**0.12**	**-2.45**	**0.014**
Trillium	**Intercept**	**-3.32**	**0.51**	**-6.48**	**<0.001**
	Initial size	**0.93**	**0.06**	**16.59**	**<0.001**
	Deer	1.04	0.65	1.60	0.110
	Earthworm	0.73	0.61	1.20	0.230
	Year	**1.66**	**0.12**	**13.34**	**<0.001**
	Deer x Earthworm	**-1.93**	**0.88**	**-2.20**	**0.028**
	Year x Deer	**-0.42**	**0.18**	**-2.40**	**0.017**
	Year x Earthworm	-0.19	0.17	-1.10	0.273
	Deer x Year x Earthworm	**0.70**	**0.25**	**2.78**	**0.005**

Appendix III.6. Random effects of site and plot within site from Survival, Height, Width and Insect Attack models.

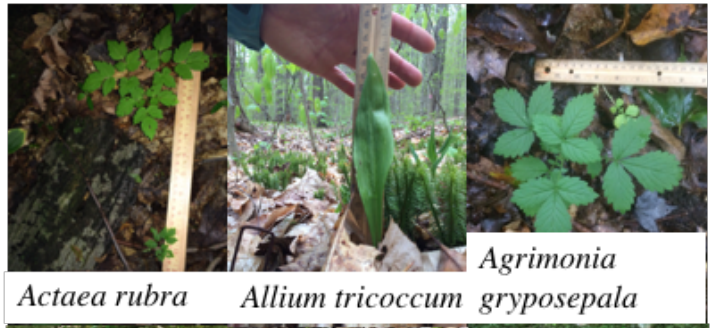
		Survival		Height		Width		Insect attack	
		Variance	**SD**	**Variance**	**SD**	**Variance**	**SD**	**Variance**	**SD**
Actaea	Site [plot]	0.03	0.16	0.00	0.02	0.51	0.71	0.00	0.00
	Site	0.31	0.56	0.18	0.43	0.00	0.00	0.26	0.51
	Residual			4.17	2.04	11.95	3.46		
Adiantum	Site [plot]	0.00	0.00	1.49	1.22	0.00	0.00	0.00	0.00
	Site	0.03	0.17	1.58	1.26	1.24	1.11	0.05	0.23
	Residual			44.21	6.65	17.40	4.17		
Allium	Site [plot]	0.00	0.00	0.00	0.00	0.01	0.07	0.00	0.00
	Site	0.44	0.67	0.00	0.00	0.00	0.00	0.00	0.00
	Residual			9.09	3.01	0.27	0.52		
Agrimonia	Site [plot]	0.08	0.27						
	Site	0.22	0.47						
	Residual								
Arisaema	Site [plot]	0.06	0.25	0.00	0.00			0.00	0.00
	Site	0.00	0.00	0.00	0.00			0.00	0.00
	Residual			10.62	3.26				
Brachyelytrum	Site [plot]	0.02	0.14	1.76	1.33			0.04	0.21
	Site	0.06	0.25	1.30	1.14			0.05	0.23
	Residual			19.16	4.38				
Carex	Site [plot]	0.04	0.20						
	Site	0.17	0.41						
	Residual								
Caul	Site [plot]	0.02	0.15	0.02	0.14	0.11	0.33	0.00	0.00
	Site	0.04	0.21	0.23	0.48	0.03	0.18	0.00	0.00
	Residual			10.30	3.21	11.92	3.45		
Dryop	Site [plot]	0.03	0.19	5.01	2.24			0.00	0.00
	Site	0.02	0.12	0.00	0.00			0.00	0.05
	Residual			140.74	11.86				
Geum	Site [plot]	0.11	0.33						
	Site	0.31	0.56						
	Residual								
Geranium	Site [plot]	0.00	0.00						
	Site	0.07	0.26						
	Residual								
Maianthemum	Site [plot]	0.00	0.00	0.31	0.56			0.07	0.26
	Site	0.07	0.27	0.04	0.21			0.00	0.00

	Residual			8.38	2.90				
Polygonatum	Site [plot]	0.07	0.26	0.00	0.00	0.00	0.04	0.00	0.00
	Site	0.00	0.00	0.29	0.54	0.00	0.00	0.17	0.41
	Residual			11.66	3.41	1.24	1.12		
Polygonum	Site [plot]	0.12	0.34						
	Site	0.05	0.22						
	Residual								
Polystich	Site [plot]	0.11	0.33	4.54	2.13			0.00	0.05
	Site	0.25	0.50	0.85	0.92			0.00	0.00
	Residual			159.49	12.63				
Quercus	Site [plot]	0.18	0.42	0.00	0.00	0.00	0.06	0.02	0.12
	Site	0.35	0.59	0.38	0.62	0.50	0.71	0.15	0.39
	Residual			10.69	3.27	5.07	2.25		
Sang	Site [plot]	0.01	0.09			0.05	0.22	0.00	0.00
	Site	0.07	0.26			0.00	0.00	0.00	0.00
	Residual					2.35	1.53		
Thalictrum	Site [plot]	0.00	0.00	0.00	0.00			0.05	0.22
	Site	0.11	0.34	1.63	1.28			0.04	0.21
	Residual			27.85	5.28				
Tiarella	Site [plot]	0.10	0.31			0.06	0.25	0.00	0.00
	Site	0.00	0.00			0.00	0.00	0.03	0.17
	Residual					4.01	2.00		
Trillium	Site [plot]	0.03	0.17			0.10	0.32	0.00	0.00
	Site	0.17	0.41			0.25	0.50	0.00	0.00
	Residual					6.48	2.55		

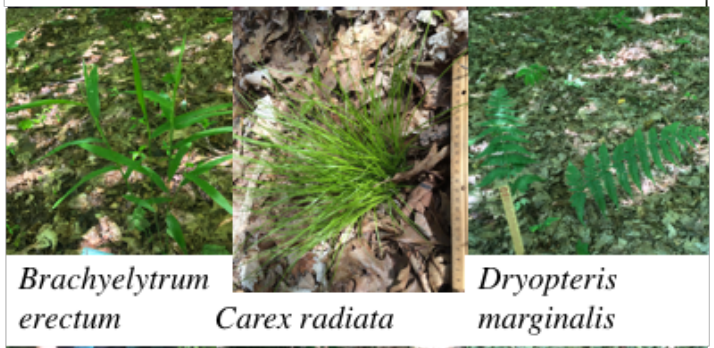
		# culms		Flowering probability	
		Variance	SD	Variance	SD
Carex	Site [plot]	92.60	9.62	0.00	0.00
	Site	172.00	13.11	0.41	0.64
	Residual	718.80	26.81		

Appendix III.7. A selection of species of seedlings established in five forested sites in central New York. Earthworm impact is categorized by the cumulative impact by 2017 (the end of the experiment).

Benefit from earthworms



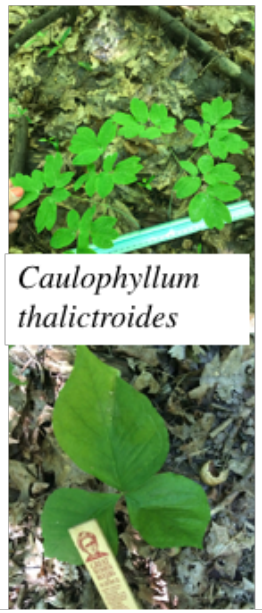
Actaea rubra *Allium tricoccum* *Agrimonia gryposepala*



Brachyelytrum erectum *Carex radiata* *Dryopteris marginalis*

Geum canadense *Thalictrum dicocum* *Polystichum acrostichoides*

Unaffected



Caulophyllum thalictroides

Arisaema tryphyllum

Negatively affected by earthworms

Maianthemum canadense *Trillium erectum*

Tiarella cordifolia *Polygonatum biflorum*

Appendix III.8. Number of flowering transplants in forested plots in central NY at the end of the experiment (2017). Only angiosperm species with at least one flowering individual was included.

Species	- Earthworm - Deer	+ Earthworm - Deer	- Earthworm + Deer	+ Earthworm + Deer
Actaea rubra	0	0	0	1
Agrimonia gryposepala	0	1	0	0
Arisaema triphyllum	0	2	0	2
Brachyelytrum erectum	0	2	1	6
Carex radiata	3	18	0	9
Geum canadense	0	1	0	0
Maianthemum racemosum	0	2	0	0
Polygonatum biflorum	1	3	0	1
Sanguinaria canadensis	0	7	0	0
Thalictrum dioicum	0	2	0	1
Tiarella cordifolia	3	9	2	0
Trillium erectum	1	1	0	0

Appendix III.9. Model output for GLMMs with Binomial distribution for transplant survival (n = 31 - 282 per species at end of experiment) in forested plots in central NY from 2012 - 2017. Models include random effects of site and plot within site reported in Appendix III.6.

Species	Parameter	Estimate	SE	Z	p
Actaea	**Intercept**	**-3.74**	**0.50**	**-7.43**	**<0.001**
	Initial size	-0.05	0.07	-0.72	0.474
	Deer	**1.37**	**0.49**	**2.82**	**0.005**
	Earthworm	**1.32**	**0.32**	**4.10**	**<0.001**
	Year	**0.50**	**0.09**	**5.57**	**<0.001**
	Deer x Earthworm	**-0.89**	**0.42**	**-2.13**	**0.033**
	Year x Deer	**-0.26**	**0.13**	**-1.99**	**0.047**
Adiantum	**Intercept**	**-3.20**	**0.58**	**-5.47**	**<0.001**
	Initial size	0.04	0.05	0.81	0.417
	Deer	0.35	0.69	0.51	0.610
	Earthworm	**1.83**	**0.55**	**3.31**	**0.001**
	Deer x Earthworm	-0.05	0.74	-0.07	0.943
Allium	**Intercept**	**-2.45**	**0.76**	**-3.23**	**0.001**
	Initial size	-0.03	0.05	-0.54	0.589
	Deer	-0.22	0.62	-0.35	0.726
	Earthworm	0.86	0.49	1.77	0.077
	Deer x Earthworm	-0.45	0.77	-0.58	0.559
Arisaema	**Intercept**	**-2.53**	**0.50**	**-5.11**	**<0.001**
	Initial size	0.03	0.06	0.61	0.543
	Deer	-1.50	0.81	-1.84	0.066
	Earthworm	**1.32**	**0.43**	**3.08**	**0.002**
	Deer x Earthworm	0.97	0.87	1.12	0.264
Brachyelytrum	Intercept	-1.41	0.78	-1.80	0.071
	Initial size	-0.17	0.11	-1.57	0.117
	Deer	-0.38	0.59	-0.64	0.524
	Earthworm	0.80	0.46	1.74	0.082
	Deer x Earthworm	-0.01	0.74	-0.01	0.992
Caulophyllum	**Intercept**	**-1.69**	**0.47**	**-3.60**	**<0.001**
	Initial size	0.07	0.04	1.67	0.095
	Deer	-0.19	0.40	-0.46	0.646
	Earthworm	**0.78**	**0.35**	**2.22**	**0.026**

	Deer x Earthworm	-0.29	0.48	-0.61	0.541
Dryopteris	**Intercept**	**-2.19**	**0.58**	**-3.78**	**<0.001**
	Initial size	0.01	0.05	0.19	0.848
	Deer	-0.41	0.46	-0.88	0.377
	Earthworm	**0.83**	**0.35**	**2.39**	**0.017**
	Deer x Earthworm	0.01	0.56	0.01	0.989
Maianthemum	**Intercept**	**-1.74**	**0.15**	**-11.82**	**<0.001**
	Initial size	0.00	0.00	-0.44	0.657
	Deer	-0.15	0.20	-0.73	0.467
	Earthworm	**0.37**	**0.18**	**2.05**	**0.040**
	Deer x Earthworm	0.37	0.26	1.44	0.149
Polygonatum	**Intercept**	**-2.73**	**0.43**	**-6.39**	**<0.001**
	Initial size	**0.14**	**0.05**	**2.96**	**0.003**
	Deer	0.60	0.43	1.37	0.170
	Earthworm	**1.34**	**0.40**	**3.38**	**0.001**
	Deer x Earthworm	**-1.43**	**0.58**	**-2.48**	**0.013**
Polystichum	**Intercept**	**-2.97**	**0.34**	**-8.73**	**<0.001**
	Initial size	0.03	0.03	1.25	0.212
	Deer	**0.67**	**0.28**	**2.42**	**0.015**
	Earthworm	**1.17**	**0.27**	**4.26**	**<0.001**
	Deer x Earthworm	-0.55	0.35	-1.57	0.116
Quercus	**Intercept**	**-1.36**	**0.18**	**-7.48**	**<0.001**
	Initial size	0.00	0.00	0.68	0.495
	Deer	-0.05	0.22	-0.24	0.810
	Earthworm	**0.49**	**0.20**	**2.48**	**0.013**
	Deer x Earthworm	0.01	0.28	0.05	0.960
Sanguinaria	Intercept	-0.73	0.45	-1.64	0.100
	Initial size	**0.08**	**0.04**	**2.39**	**0.017**
	Deer	-0.14	0.32	-0.45	0.654
	Earthworm	0.50	0.30	1.66	0.098
	Deer x Earthworm	-0.57	0.42	-1.34	0.179
Thalictrum	**Intercept**	**-3.47**	**0.67**	**-5.18**	**<0.001**
	Initial size	0.19	0.13	1.45	0.146
	Deer	-0.25	0.78	-0.32	0.749

	Earthworm	**1.37**	**0.58**	**2.36**	**0.018**
	Deer x Earthworm	-0.74	0.91	-0.81	0.416
Tiarella	**Intercept**	**-2.86**	**0.46**	**-6.22**	**<0.001**
	Initial size	-0.01	0.03	-0.33	0.741
	Deer	-0.18	0.29	-0.61	0.544
	Earthworm	0.04	0.61	0.07	0.942
	Year	**0.44**	**0.11**	**4.14**	**<0.001**
	Deer x Earthworm	0.45	0.40	1.14	0.256
	Year x Earthworm	**0.31**	**0.15**	**2.03**	**0.042**
Trillium	**Intercept**	**-2.02**	**0.24**	**-8.29**	**<0.001**
	Initial size	0.17	0.09	1.80	0.072
	Deer	0.09	0.29	0.32	0.746
	Earthworm	**0.97**	**0.27**	**3.62**	**<0.001**
	Deer x Earthworm	-0.14	0.38	-0.37	0.708

Appendix III.10. Model output for LMMs with Gaussian distribution (above) and GLMMs for flowering probability of *Carex* (n = 61 at end of experiment) in forested plots in central NY from 2012 - 2017. Models include random effects of site and plot within site reported in Appendix III.6.

Response	Parameter	Estimate	SE	Z	p
# culms	Intercept	-15.08	13.07	-1.15	0.248
	Initial size	**1.90**	**0.69**	**2.76**	**0.006**
	Deer	-11.43	8.15	-1.40	0.161
	Earthworm	-20.61	12.99	-1.59	0.113
	Year	2.11	2.53	0.83	0.404
	Deer x Earthworm	9.45	6.60	1.43	0.152
	Year x Earthworm	**10.95**	**3.00**	**3.65**	**<0.001**
Flowering probability	**Intercept**	**-6.82**	**0.95**	**-7.21**	**<0.001**
	Initial size	**0.14**	**0.05**	**2.56**	**0.010**
	Deer	1.23	0.83	1.47	0.140
	Earthworm	0.07	0.89	0.07	0.941
	Year	**0.80**	**0.20**	**4.06**	**<0.001**
	Deer x Earthworm	0.10	0.63	0.15	0.877
	Year x Earthworm	**0.44**	**0.20**	**2.23**	**0.026**
	Year x Deer	**-0.37**	**0.18**	**-2.09**	**0.037**